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(54) **Neuroprotektív biciklusos vegyületek és ezeket tartalmazó gyógyszerkészítmények**

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(54) NEUROPROTECTIVE BICYCLIC COMPOUNDS AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM

NEUROPROTEKTIVE BICYCLISCHE VERBINDUNGEN UND PHARMAZEUTISCHE ZUSAMMENSETZUNGEN, DIE DIESE ENTHALTEN

COMPOSES NEUROPROTECTEURS BICYCLIQUES ET PROCEDES D'UTILISATION CORRESPONDANTS

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(56) References cited:
WO-A-99/40931 **WO-A-03/041655**
DE-A1- 1 620 386

- **K. R. C. PRAKASH ET AL: "Synthesis and Biological Activity of Novel Neuroprotective Diketopiperazines" BIOORGANIC AND MEDICINAL CHEMISTRY, vol. 10, no. 9, 2002, pages 3043-3048, XP002311146**

EP 1 664 050 B1

Description

Field of the Invention

[0001] The present invention relates to novel bicyclic compounds structurally related to diketopiperazines. More particularly, this invention relates to these compounds and pharmaceutical compositions thereof for use in the treatment of diseases and conditions characterised by neuronal degeneration and/or death.

BACKGROUND

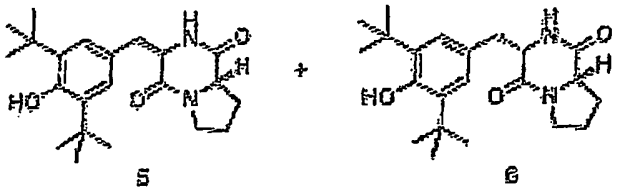
[0002] Degeneration and/or death of cells in the nervous system are major factors in many diseases and medical conditions. Such diseases and conditions include traumatic brain and spinal cord injuries, stroke, neural perfusion secondary to cardiac arterial bypass graft surgery (CABG), Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis and other neurodegenerative diseases. It is of interest to prevent or decrease such cell death and degeneration.

[0003] Certain compounds are useful as neuroprotective agents. One such compound is insulin-like growth factor 1 (IGF-1) (Scheepens et al, WO00/13650). IGF-1 is a naturally occurring peptide that can decrease the binding of glutamate to the glutamate receptors of neurons (Bourguinon, US Patent No. 5,804,550). IGF-1 can also decrease neuronal degradation caused by damage and disease. IGF-1 is cleaved by proteolysis *in vivo* to give des_{1-3} IGF-1 and the N-terminal tripeptide Gly-Pro-Glu (GPE). GPE and analogues have been found to be neuroprotective (Gluckman et al, US Patent No. 6,187,906 incorporated herein by reference).

[0004] However, such peptides are far from ideal for the treatment of neural death and degeneration especially as they are rapidly metabolised *in vivo.* There is a need for compounds that provide neuroprotective and neuroregenerative properties and are more metabolically stable especially as regards resistance to proteases.

[0005] A derivative of GPE; cyclic Pro-Gly ("cPG"), a diketopiperazina, has been shown to be neuroprotective and neuroregenerative. cPG was found to prevent toxic neural degeneration and cell death and to promote neurite outgrowth in neurons (Guan et al, PCT/US02/36235). Diketopiperazine analogues of thyrotropin-releasing hormone (TRH) are known to be neuroprotective (Kozikowski et al WO99/40931).

K.R.C. Prakash et al., " Synthesis and Biological Activity of Novel Neuroprotective Diketopiperazines", Bioorganic & Medicinal Chemistry, Volume 10, Issue 9, September 2002, Pages 3043-3048, disclose diketopiperazines of the formulae:



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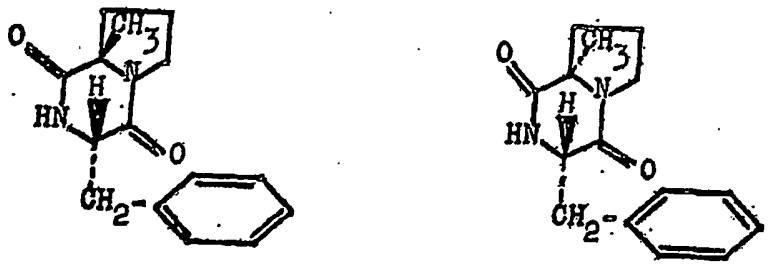
said to prevente neuronal death in an in vitro model of traumatic injury The neuroprotective profile of these compounds suggests that they may be useful as treatments for neuronal degeneration in vivo, potentially through several different mechanisms. DE 1620386 A1 discloses the compounds of formulae:

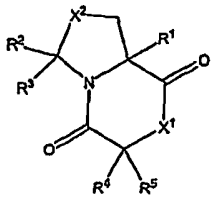


as intermediates for preparing alkaloids useful in the treatment of migraine.

SUMMARY

[0006] Embodiments of this invention include novel diketopiperazines that are structurally related to cPG.

[0007] One aspect of this invention provides novel cyclic compounds having the structural formulas and substituents described below.



Formula 1

[0008] In some aspects, compounds of Formula 1 include substituents where:

X^1 is selected from the group consisting of NR', O and S;
X^2 is selected from the group consisting of CH_2, NR', O and S;
R1 is selected from the group consisting of, -OR',-SR',-NR'R',-NO2.-CN,-C (O) R', -C (O) OR', -C (O) NR'R', -C (NR') NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl : each R'is independently selected from the group consisting of-H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl
R2. R3. R4 and R5 are independently selected from the group consisting of H,-OR',-SR',-NR'R',-NO2,-CN,-C (O) R', -C (O) OR', -C (O) NR'R', -C (NR') NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl: each R'is independently selected from the group consisting of-H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl :
or R4 and R5 taken together are-CH2-(CH2) n-CH2- where n is an integer from 0-6;
or R2 and R3 taken together are-CH2- (CH2) n-CH2- where n is an integer from 0-6:
with the proviso that when R1= methyl and R2=R3=R4=H then Rs # benzyl,

wherein substituted alkyl, substituted heteroalkyl, substituted alkenyl, substituted . alkynyl, substituted aryl, substituted heteroaryl or substituted arylalkyl are alkyl, heteroalkyl, alkenyl, alkynyl, aryl, heteroaryl or arylalkyl radical wherein one or more of the hydrogen atoms are independently replaced with another substituent including -R'.-OR',-SR',-NR'R',-NO2, -CN,-C (O) R', -C (O) OR', -C (O) NR'R', -C (NR') NR'R',-NR'-C (NR')-OR',-NR'-C(NR')-SR', NR'-C (NR')-NR'R', trihalomethyl and halogen where each R'is independently-H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl or heteroarylalkyl.

[0009] Other aspects of the invention provides pharmaceutically acceptable salts of the compounds described in Formula 1.

[0010] In still other aspects, this invention provides pharmaceutical compositions comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of at least one compound of this invention. These compositions find use as anti-apoptotic and anti-necrotic agents and for other conditions involving neural degeneration or injury.

[0011] In further aspects, this invention provides the compounds for use in methods of treating an animal having a disease or injury capable of treatment by administration of a suitable compound of Formula 1, comprising administration to that animal of at least one compound of this invention to decrease neurodegeneration caused by an injury or disease. In certain other aspects, this disclosure includes methods for treating an animal with a diketopiperazine of any of Formula 1 in conjunction with at least one other conventional therapeutic agent for the disease being treated.

[0012] In yet further aspects, the animal to be treated is a human.

[0013] This disclosure provides methods of synthesizing, formulating and preparing pharmaceutical preparations comprising compounds of Formula 1 of this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] This invention is described with reference to specific embodiments thereof. Other aspects of this invention can be appreciated with reference to the drawings, in which:

Figure 1 is a graph showing effects of cyclic G-2allylP on neuronal survival in animals following excitotoxic oxidative stress.

Figure 2 is a graph showing effects of cyclic cyclopentylG-2MeP on neuronal survival in animals following excitotoxic oxidative stress.

Figure 3 is a graph showing the neuroprotective effects of cyclic G-2allylP in animals subjected to global brain ischaemia.

Figure 4 is a graph showing effects of different doses of cyclic G-2allylP on neuroprotection in animals subjected to global brain ischaemia.

DETAILED DESCRIPTION

Definitions

[0015] "Alkenyl" refers to an unsaturated branched, straight chain or cyclic hydrocarbon radical having at least one carbon-carbon double bond. The radical may be in either the *cis* or *trans* conformation about the double bond(s). Exemplary alkenyl groups include allyl, ethenyl, propenyl, isopropenyl, butenyl, isobutenyl, cyclopentenyl and the like. In some embodiments the alkenyl groups are (C_2-C_6) alkenyl, and in other embodiments, allyl can be particularly useful.

[0016] "Alkyl" refers to a saturated branched, straight chain or cyclic hydrocarbon radical. Exemplary alkyl groups include methyl, ethyl, isopropyl, cyclopropyl, *tert*-butyl, cyclopropylmethyl, hexyl and the like. In some embodiments the alkyl groups are (C_1-C_6) alkyl.

[0017] "Alkynyl" refers to an unsaturated branched, straight chain or cyclic hydrocarbon radical having at least one carbon-carbon triple bond. Exemplary alkynyl groups include ethynyl, propynyl, butynyl, isobutynyl and the like. In some embodiments the alkynyl group is (C_2-C_6) alkynyl.

[0018] "Aryl" refers to an unsaturated cyclic hydrocarbon radical with a conjugated π electron system. Exemplary aryl groups include phenyl, naphthyl and the like. In some embodiments the aryl group is (C_5-C_{20}) aryl.

[0019] "Arylalkyl" refers to a straight chain alkyl, alkenyl or alkynyl group wherein one of the hydrogen atoms bound to the terminal carbon is replaced with an aryl group. Exemplary arylalkyl groups include benzyl, naphthylmethyl, benzylidene and the like.

[0020] "Growth factor" refers to an extracellularly active polypeptide that stimulates a cell to grow or proliferate by interacting with a receptor on the cell.

[0021] "Heteroalkyl" refers to an alkyl moiety wherein one or more carbon atoms are replaced with another atom such as N, P, O, S etc. Exemplary heteroalkyl groups include pyrrolidine, morpholine, piperidine, piperazine, imidazolidine, pyrazolidine, tetrahydrofuran, (C_1-C_{10}) substituted amines, (C_2-C_6) thioethers and the like.

[0022] "Heteroaryl" refers to an aryl moiety wherein one or more carbon atoms are replaced with another atom such as N, P, O, S etc. Exemplary heteroaryl groups include carbazole, furan, imidazole, indazole, indole, isoquinoline, purine, pyrazine, pyrazole, pyridazine, pyridine, pyrrole, thiazole, thiophene, triazole and the like.

[0023] "Injury" includes any acute or chronic damage of an animal that results in degeneration or death of cells in the nervous system. Such cells include neuronal cells and non-neuronal cells. Injury includes stroke, non-hemorrhagic stroke, traumatic brain injury, perinatal asphyxia associated with fetal distress such as following abruption, cord occlusion or associated with intrauterine growth retardation, perinatal asphyxia associated with failure of adequate resuscitation or respiration, severe CNS insults associated with near miss drowning, near miss cot death, carbon monoxide inhalation, ammonia or other gaseous intoxication, cardiac arrest, coma, meningitis, hypoglycemia and status epilepticus, episodes of cerebral asphyxia associated with coronary bypass surgery, hypotensive episodes and hypertensive crises, and cerebral trauma. It is to be understood that the above examples are by way of illustration only.

[0024] A "pharmaceutically acceptable excipient" refers to an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients may be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous.

[0025] A "pharmaceutically acceptable salt" refers to a salt that is pharmaceutically acceptable and has the desired pharmacological properties. Such salts include salts that may be formed where acidic protons present in the compounds are capable of reacting with inorganic or organic bases. Suitable inorganic salts include those formed with the alkali metals, e.g. sodium and potassium, magnesium, calcium, and aluminium. Suitable organic salts include those formed with organic bases such as the amine bases e.g. ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Such salts also include acid addition salts formed with inorganic acids (e.g. hydrochloric and hydrobromic acids) and organic acids (e.g. acetic acid, citric acid, maleic acid, and the alkane- and arene-sulfonic

acids such as methanesulfonic acid and benzenesulfonic acid). When there are two acidic groups present, a pharmaceutically acceptable salt may be a mono-acid mono-salt or a di-acid salt; and similarly where there are more than two acidic groups present, some or all of such groups can be present as salts.

[0026] A "protecting group" has the meaning conventionally associated with it in organic synthesis, i.e. a group that selectively blocks one or more reactive sites in a multifunctional compound such that a chemical reaction can be carried out selectively on another unprotected reactive site and such that the group can readily be removed after the selective reaction is complete.

[0027] "Substituted" refers to where one or more of the hydrogen atoms on an alkyl, heteroalkyl, alkenyl, alkynyl, aryl, heteroaryl or arylalkyl radical are independently replaced with another substituent. Substituents include -R', -OR', -SR', NR'R', $-NO_2$, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', -NR'-C(NR')-OR', -NR'-C(NR')-SR', NR'-C(NR')-NR'R', trihalomethyl and halogen where each R' is independently -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl or heteroarylalkyl.

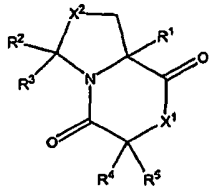
[0028] A "therapeutically effective amount" means the amount that, when administered to an animal for treating a disease, is sufficient to effect treatment for a disease or an injury. A "therapeutically effective amount" means an amount that decreases adverse symptoms or findings, promotes desirable symptoms or findings, and/or treats an underlying disorder, and/or is curative.

[0029] "Treating" or "treatment" of a disease includes preventing the disease from occurring in an animal that may be predisposed to the disease but does not yet experience or exhibit symptoms of the disease (prophylactic treatment), inhibiting the disease (slowing or arresting its development), providing relief from the symptoms or side-effects of the disease (including palliative treatment), and relieving the disease (causing regression of the disease).

[0030] Implicit hydrogen atoms (such as the hydrogens on the pyrrole ring, etc.) are omitted from the formulae for clarity, but should be understood to be present.

Compounds of the Invention

[0031] Certain embodiments of this invention include novel derivatives of cPG having structures as described below for Formulas 1-7.



Formula 1

[0032] In certain embodiments, compounds of Formula 1 include substituents where:

X^1 is selected from the group consisting of NR', O and S;
X^2 is selected from the group consisting of CH_2, NR', O and S;
R1 is selected from the group consisting of, -OR',-SR',-NR'R',-NO2,-CN,-C (O) R', -C(O) OR', -C (O) NR'R', -C (NR') NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroakylalkyl and substituted heteroarylalkyl ; each R'is independently selected from the group consisting of-H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl
R2, R3, R4 and R5 are independently selected from the group consisting of- H,-OR',-SR',-NR'R',-NO2,-CN,-C (O) R', -C (O) OR', -C (O) NR'R', -C (NR') NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl : each R'is independently selected from the group consisting of-H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl;
or R4 and R5 taken together are-CH2- (CH2) n-CH2- where n is an integer from 0-6;
or R2 and R3 taken together are-CH2- (CH2) n-CH2- where n is an integer from 0-6:
with the proviso that when R1= methyl and R2=R3=R4=H then Rs # benzyl,

wherein substituted alkyl, substituted heteroalkyl, substituted alkenyl, substituted alkynyl, substituted aryl, substituted heteroaryl or substituted arylalkyl are alkyl, heteroalkyl, alkenyl, alkynyl, aryl, heteroaryl or arylalkyl radical wherein one

or more of the hydrogen atoms are independently replaced with another substituent including -R',-OR',-SR',-NR'R',-NO2, -CN,-C (O) R', -C (O) OR', -C (O) NR'R', -C (NR') NR'R',-NR'-C (NR')-OR',-NR'- C (NR') -SR', NR'-C (NR')-NR'R', trihalomethyl and halogen where each R'is independently-H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl or heteroarylalkyl.

[0033] In further embodiments, compounds of Formula 1 include substituents where:
R^1=methyl, R^2=R^3=R^4=R^5= H, X^1=NH, X^2=CH_2;
R^1=allyl, R^2=R^3=R^4=R^5=H, X^1=NH, X^2=CH_2;
R^1=R^2=R^3=H, R^4=R^5=methyl, X^1=NH, X^2=CH_2;
R^1=R^4=R^5 =H, R^2=R^3=methyl, X^1=NH, X^2=CH_2.

[0034] In other embodiments of the invention, compounds of Formula 1 include substituents where;
R^4 and R^5 taken together are -CH_2-$(CH_2)_n$-CH_2- and:
R^1=methyl, R^2=R^3=H, n=0, X^1=NH, X^2=CH_2;
R^1=methyl, R^2=R^3=H, n=2, X^1=NH, X^2=CH_2;
R^1=allyl, R^2=R^3=H, n=0, X^1=NH, X^2=CH_2;
R^1=allyl, R^2=R^3=H, n=2, X^1=NH, X^2=CH_2.
R^1=methyl, R^2=R^3=H n=3, X^1=NH, X^2=CH^2;
R^1=allyl, R^2=R^3=H, n=3, X^1=NH, X^2=CH_2.

[0035] In still other embodiments of the invention, compounds of Formula 1 include substituents where R^1=methyl or allyl, R^2=R^3=R^4=H and R^5 is selected from the group consisting of the side chains of the amino acids: alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, proline, serine, threonine, tryptophan, tyrosine, valine, norvaline, norleucine, citruline, ornithine, homocysteine, homoserine, alloisoleucine, isovaline, sarcosine and the like.

[0036] In yet further embodiments of the invention, compounds of Formula 1 include substituents where:

R^1=methyl, R^2=R^3=methyl, R^4=R^5=H, X^1=NH and X^2=S;
R^1=allyl, R^2=R^3=methyl, R^4=R^5=H, X^1NH and X^2=S.

[0037] Those with skill in the art will appreciate that the above structural representations can contain chiral centres, the number of which will depend on the different substituents. The chirality may be either R or S at each centre. The structural drawings can represent only one of the possible tautomeric, conformational isomeric or enantiomeric forms, and it should be understood that the invention encompasses any tautomeric, conformational isomeric or enantiomeric form which exhibit biological or pharmacological activity as described herein.

Pharmacology and Utility

[0038] Certain aspects of this invention include the use of compounds of the invention for use in treatment or prevention of cell damage, degeneration and/or death in mammals in response to injury or disease. Some embodiments of the disclosure comprise delivering a composition containing a compound of the invention to an animal suffering from neural degeneration, and in some cases, conditions involving apoptotic and necrotic cell death. In some embodiments, compositions are desirable to treat an injury or disease of the CNS affecting or liable to affect brain cells. Compositions are provided that can also include one or more other agents that promote neural regeneration, decrease cell degeneration or death, or are neuroprotective.

[0039] Such other agents may be selected from the group consisting of for example, growth factors and associated derivatives, e.g., insulin-like growth factor-I [IGF-I], insulin-like growth factor-II [IGF-II], the tripeptide GPE, transforming growth factor-ß1, activin, growth hormone, nerve growth factor, growth hormone binding protein, and/or IGF-binding proteins.

[0040] Other aspects of the disclosure include compositions and methods of promoting fasiculation of axons. By promoting formation of nerve bundles, compounds of the invention may be useful in treating conditions in which nerve processes (axons and/or dendrites) have become severed, such as in sharp force injuries, local areas of necrosis or disease, or other localized injuries to nerve processes.

[0041] In yet other aspects, compositions and methods to treat or prevent cell damage and death in response to injury and disease, including CNS injury and disease, comprise administration of a therapeutic amount of a compound of the invention alone or in combination with other agents, after the insult. These aspects can be particularly desirable in situations of unexpected injury, such as in cardiac arrest, trauma such as head injuries caused by automobile accidents, head wounds and the like.

[0042] In still further embodiments, of the disclosure compounds of the invention can be used either alone or in combination with other agents to prevent adverse effects of planned brain injury. Such conditions include CABG or other planned surgeries such as brain surgery, vascular surgery or other interventions that may lead to decreased perfusion

of the nervous system. By treating an animal, such as a human being, in advance and/or simultaneously and/or after the surgery, adverse neurological effects can be ameliorated.

[0043] As indicated above, the present invention is broadly based upon the applicant's finding that compounds of the invention can protect cells, particularly nerve cells, against damage, loss of neurites, and/or apoptotic or necrotic cell death.

[0044] It is herein demonstrated that compounds of the invention exhibit neuroprotection in both cell culture and in animal models of neurodegenerative disease and can therefore be an effective addition or alternative to conventional therapies for neural degeneration.

[0045] Although the mechanism of the protective effects is not known, one possible mechanism involves protecting cells from apoptotic and necrotic cell death. However, regardless of the mechanism of action, compounds of the invention can be used as an effective therapy for a variety of neurological diseases, including hypoxia, ischemia and neurotoxin-induced nerve damage. Moreover, compounds of the invention can be used in the absence of any particular neurological deficit to promote neurite outgrowth and fasiculation of nerves. Thus, in situations in which cell death is not necessarily associated with the neurological disorder (e.g., axonal damage such as caused by spinal cord injury), administration of compounds of the invention may be an effective way of promoting neurite regeneration.

Therapeutic Applications

[0046] Compositions of the invention find use in the treatment of animals, such as human patients, suffering from neural injury or disease. Still more generally, the compositions of the invention find use in the treatment of mammals, such as human patients, suffering from nerve damage or potential apoptotic and/or necrotic cell death, due to injuries and diseases.

[0047] Specific conditions and diseases characterised by neuronal degeneration, apoptosis and/or necrosis include Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, spinal muscular atrophy, peripheral neuropathy, Creutzfeldt-Jakob disease, AIDS dementia, progressive supranuclear palsy, myelinopathia centralis diffusa (vanishing white matter disease), chronic neurodegenerative disease, Huntington's disease, stroke, ischemic injury, hypoxic injury, reperfusion injury, head injury, CNS trauma, epilepsy, cerebral ischemia, glaucoma, retinal disorders, optic neuropathy, optic neuritis, Down's syndrome, encephalomyelitis, meningitis, panencephalitis, neuroblastoma, schizophrenia and depression. Each of the above conditions exhibits pathophysiological findings and symptoms that are mimicked by neurotoxicity associated with glutamate toxicity.

[0048] Still more generally, the compounds of the invention have application in the induction of nerve bundle formation following insult in the form of trauma, toxin exposure, asphyxia or hypoxia-ischemia. Additionally, the compounds of the invention have application in the treatment or prevention of apoptosis in response to injury or disease in the form of cancers, viral infections, autoimmune diseases, neurological diseases and injuries and cardiovascular diseases.

[0049] Treatment may be given before an injury, for example, before elective surgery. Examples of relevant elective procedures include neural surgery, in which retraction of lobes of the brain may lead to cerebral oedema, or heart operations, such as valve replacement, in which inevitable small emboli are said to lead to detectable impairment of brain function in some 75% of cases.

Determining Efficacy

[0050] The anti-apoptotic and anti-necrotic activity of compounds of the invention on cellular death can be measured *in vivo* using cell counts by methods known to those skilled in the art including the methods of Klempt *et al* (Klempt et al, 1992, Molecular Brain Research: 13: 93-101), microscopic examinations of morphology, cell counts of surviving and dead neurons stained with thionin/fuchsin and the like. Compounds of the invention can also be measured *in vitro* using mass spectroscopy, immunological, or chromatographic methods known in the art.

[0051] CNS damage may for example be measured clinically by the degree of permanent neurological deficit cognitive function, and/or propensity to seizure disorders. Herein are disclosed histological techniques suitable for measuring effects *in vivo.*

[0052] The therapeutic ratio of a compound is understood to be the ratio of (1) the mean dose that causes adverse side effect over (2) the mean dose that causes a desirable therapeutic effect. Thus, for compounds for which have therapeutic effects at relatively low doses and undesirable side effects at high doses, the therapeutic ratio is >1. Therapeutic ratio can be determined, for example, by comparing the dose that produces significant weight loss (or other observable side-effect) divided by the dose that produces anti-apoptotic and anti-necrotic activity in a suitable *in vivo* animal species such as the rat or mouse. Suitable animal systems useful for determining therapeutic effects of compounds of this invention include hypoxic-ischemic injury (Sirimanne et al, 1994 Journal of Neuroscience Methods: 55: 7-14), experimental immune encephalomyelitis (Mendel et al., 1995 Eur. J. Immunol.: 25: 1951-1959) and glutamate toxicity.

Pharmaceutical Compositions and Administration

[0053] Compounds of the invention can be administered as part of a medicament or pharmaceutical preparation. This can involve combining a compound of the invention with any pharmaceutically appropriate carrier, adjuvant or excipient. The selection of the carrier, adjuvant or excipient will of course usually be dependent upon the route of administration to be employed.

[0054] In general, compounds of this invention can be administered in therapeutically effective amounts by any of the usual modes known in the art, either singly or in combination with other conventional therapeutic agents for the disease being treated. A therapeutically effective amount may vary widely depending on the disease or injury, its severity, the age and relative health of the animal being treated, the potency of the compound(s), and other factors. As anti-apoptotic and anti-necrotic agents, therapeutically effective amounts of compounds of this invention may range from 0.001 to 100 milligrams per kilogram mass of the animal, with lower doses such as 0.001 to 0.1 mg/kg being appropriate for administration through the cerebrospinal fluid, such as by intracerebroventricular administration, and higher doses such as 1 to 100 mg/kg being appropriate for administration by methods such as oral, systemic (e.g. transdermal), or parenteral (e.g. intravenous) administration. A person of ordinary skill in the art will be able without undue experimentation, having regard to that skill and this disclosure, to determine a therapeutically effective amount of a compound of this invention for a given disease or injury.

[0055] Compounds of the invention may be administered peripherally *via* any peripheral route known in the art. These can include parenteral routes for example injection into the peripheral circulation, subcutaneous, intraorbital, ophthalmic, intraspinal, intracistermal, topical, infusion (using e.g. slow release devices or minipumps such as osmotic pumps or skin patches), implant, aerosol, inhalation, scarification, intraperitoneal, intracapsular, intramuscular, intranasal, oral, buccal, transdermal, pulmonary, rectal or vaginal. The compositions can be formulated for parenteral administration to humans or other mammals in therapeutically effective amounts (e.g. amounts which eliminate or reduce the patient's pathological condition) to provide therapy for the neurological diseases described above.

[0056] Desirably, if possible, when administered as anti-apoptotic and anti-necrotic agents, compounds of this invention will be administered orally. The amount of a compound of this invention in the composition may vary widely depending on the type of composition, size of a unit dosage, kind of excipients, and other factors well known to those of ordinary skill in the art. In general, the final composition may comprise from 0.0001 percent by weight (% w) to 10% w of the compound of this invention, preferably 0.001% w to 1% w, with the remainder being the excipient or excipients.

[0057] Other convenient administration routes include subcutaneous injection (e.g. dissolved in a physiologically compatible carrier such as 0.9% sodium chloride) or direct administration to the CNS. Using stereotactic devices and accurate maps of an animals' CNS, a compound may be injected directly into a site of neural damage. Such routes of administration may be especially desired in situations in which perfusion of that location is compromised either by decreased vascular perfusion or by decreased cerebral spinal fluid (CSF) flow to that area. Examples include administration by lateral cerebroventricular injection or through a surgically inserted shunt into the lateral cerebroventricle of the brain of the patient, intraveneously, direct injection into the desired location or other routes.

[0058] The effective amount of compound in the CNS may be increased by administration of a pro-drug form of a compound which comprises a compound of the invention and a carrier, where the carrier is joined to a compound of the invention by a linkage which is susceptible to cleavage or digestion within the patient. Any suitable linkage can be employed which will be cleaved or digested following administration.

[0059] However, there is no intention on the part of the applicants to exclude other forms of administration.

[0060] In further embodiments of the disclosure restoring nerve function in an animal can comprise administering a therapeutic amount of a compound of the invention in combination with another neuroprotective agent, selected from, for example, growth factors and associated derivatives (insulin-like growth factor-I [IGF-I], insulin-like growth factor-II [IGF-II], transforming growth factor-β1, activin, growth hormone, nerve growth factor, growth hormone binding protein, IGF-binding proteins [especially IGFBP-3], basic fibroblast growth factor, acidic fibroblast growth factor, the hst/Kfgk gene product, FGF-3, FGF-4, FGF-6, keratinocyte growth factor, androgen-induced growth factor. Additional members of the FGF family include, for example, int-2, fibroblast growth factor homologous factor-1 (FHF-1), FHF-2, FHF-3 and FHF-4, karatinocyte growth factor 2, glial-activating factor, FGF-10 and FGF-16, ciliary neurotrophic factor, brain derived growth factor, neurotrophin 3, neurotrophin 4, bone morphogenetic protein 2 [BMP-2], glial-cell line derived neurotrophic factor, activity-dependant neurotrophic factor, cytokine leukaemia inhibiting factor, oncostatin M, interleukin), α-, β-, γ-, or consensus interferon, and TNF-α. Other forms of neuroprotective therapeutic agents include, for example, clomethiazole; kynurenic acid, Semax, tacrolimus, L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, andrenocorticotropin-(4-9) analogue [ORG 2766] and dizolcipine [MK-801], selegiline; glutamate antagonists such as, NPS1506, GV1505260, MK-801, GV150526; AMPA antagonists such as 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX), LY303070 and LY300164; antiinflammatory agents directed against the addressin MAdCAM-1 and/or its integrin α4 receptors (α4β1 and α4β7), such as anti-MAdCAM-lmAb MECA-367 (ATCC accession no. HB-9478).

[0061] A compound is suitably administered by a sustained-release system. Suitable examples of sustained-release

compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919; EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., 1983, Biopolymers: 22: 547-56), poly(2-hydroxyethyl methacrylate) (Langer et al., 1981, J. Biomed. Mater. Res.: 15: 267), ethylene vinyl acetate (Langer et al., 1981, J. Biomed Mater. Res.: 15: 267), or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include a liposomally entrapped compound. Liposomes containing the compound are prepared by methods known per se: DE 3,218,121, EP 52,322, EP 36,676, EP 88,046, EP 143,949, EP 142,641, Japanese Pat. Appln. 83-118008, U.S. Pat. Nos. 4,485,045 and 4,544,545, and EP 102,324. Ordinarily, the liposomes are of the small (from or about 200 to 800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol percent cholesterol, the selected proportion being adjusted for the most efficacious therapy.

[0062] For parenteral administration, in one embodiment the compound is formulated generally by mixing each at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically, or parenterally, acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation.

[0063] Generally, the formulations are prepared by contacting the compound uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, a buffered solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein.

[0064] A carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; glycine; amino acids such as glutamic acid, aspartic acid, histidine, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, trehalose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counter-ions such as sodium; non-ionic surfactants such as polysorbates, poloxamers, or polyethylene glycol (PEG); and/or neutral salts, e.g., NaCl, KCl, $MgCl_2$, $CaCl_2$, etc.

[0065] A compound is typically formulated in such vehicles at a pH of from or about 4.5 to 8. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of salts of the compound. The final preparation may be a stable liquid or lyophilized solid.

[0066] Formulations of the compound in pharmaceutical compositions can also include adjuvants. Typical adjuvants which may be incorporated into tablets, capsules, and the like are a binder such as acacia, corn starch, or gelatin; an excipient such as microcrystalline cellulose; a disintegrating agent like corn starch or alginic acid; a lubricant such as magnesium stearate; a sweetening agent such as sucrose or lactose; a flavouring agent such as peppermint, wintergreen, or cherry. When the dosage form is a capsule, in addition to the above materials, it may also contain a liquid carrier such as a fatty oil. Other materials of various types may be used as coatings or as modifiers of the physical form of the dosage unit. A syrup or elixir may contain the active compound, a sweetener such as sucrose, preservatives like propyl paraben, a colouring agent, and a flavouring agent such as cherry. Sterile compositions for injection can be formulated according to conventional pharmaceutical practice. For example, dissolution or suspension of the active compound in a vehicle such as water or naturally occurring vegetable oil like sesame, peanut, or cottonseed oil or a synthetic fatty vehicle like ethyl oleate or the like may be desired. Buffers, preservatives, antioxidants, and the like can be incorporated according to accepted pharmaceutical practice.

[0067] For injection, intraventricular administration, and other invasive routes of administration, the compounds used must be sterile. Sterility may be accomplished by any method known in the art, for example filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper able to be pierced by a hypodermic injection needle.

[0068] A pharmaceutical formulation ordinarily will be stored in unit or multi-dose containers, for example, in sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10 mL vials are filled with 5 mL of sterile-filtered 1% (w/v) aqueous solution of compound, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized compound using bacteriostatic Water-for-Injection. It can be readily appreciated that other dosage forms and types of preparations can be used, and all are considered to be part of this disclosure.

Preparation of the Compounds

[0069] Starting materials and reagents used in preparing these compounds are either available from commercial

suppliers such as Aldrich Chemical Company (Milwaukee, Wis.), Bachem (Torrance, Calif.), Sigma (St.Louis, Mo.), or are prepared by methods well known to the person of ordinary skill in the art following procedures described in such references as Fieser and Fieser's Reagents for Organic Synthesis, vols 1-17, John Wiley and Sons, New York, N.Y., 1991; Rodd's Chemistry of Carbon Compounds, vols. 1-5 and supplements, Elsevier Science Publishers, 1989; Organic Reactions, vols. 1-40, John Wiley and Sons, New York, N.Y., 1991; March J; Advanced Organic Chemistry, 4th ed. John Wiley and Sons, New York, N.Y., 1992; and Larock: Comprehensive Organic Transformations, VCH Publishers, 1989. In most instances, amino acids and their esters or amides, and protected amino acids, are widely commercially available; and the preparation of modified amino acids and their amides or esters are extensively described in the chemical and biochemical literature and thus well-known to persons of ordinary skill in the art.

[0070] Starting materials, intermediates, and compounds of this invention may be isolated and purified using conventional techniques, including filtration, distillation, crystallization, chromatography, and the like. They may be characterized using conventional methods, including physical constants and spectral data.

[0071] Compounds of this invention may be prepared by the methods described below and as given in the Examples.

[0072] Compounds of this invention are generally cyclic dipeptides (bicyclic 2,5-diketopiperazines) or analogues thereof. In general, they may be prepared by methods such as are already well-known to persons of ordinary skill in the art of peptide and modified peptide synthesis, following the reaction schemes set forth in the Figures following this specification, or by following other methods well-known to those of ordinary skill in the art of the synthesis of peptides and analogues. See for example, Bodanzsky: Principles of Peptide Synthesis, Berlin, New York: Springer-Verlag 1993.

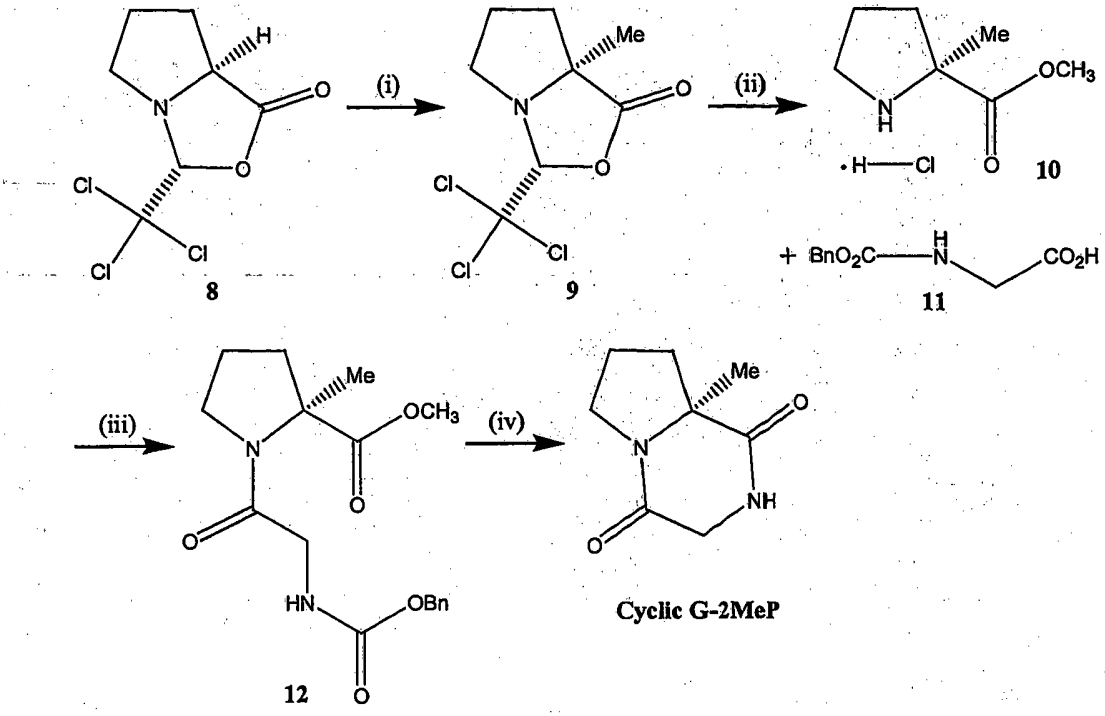
[0073] Synthesis of the diketopiperazine compounds of this invention may be by solution-phase synthesis as discussed in the Examples or *via* the solid-phase synthesis method exemplified by Merrifield et al. 1963 J. Amer. Chem. Soc.: 85, 2149-2156. Solid phase synthesis may be performed using commercial peptide synthesizers, such as the Applied Biosystems Model 430A, using the protocols established for the instrument.

[0074] Specific examples of diketopiperazine synthesis can be found in the Examples following and in, for example, Fischer, 2003, J. Peptide Science: 9: 9-35 and references therein. A person of ordinary skill in the art will have no difficulty, taking account of that skill and the knowledge available, and of this disclosure, in developing one or more suitable synthetic methods for compounds of this invention.

[0075] The choice of appropriate protecting groups for the method chosen (solid-phase or solution-phase), and of appropriate substrates if solid-phase synthesis is used, will be within the skill of a person of ordinary skill in the art. Appropriate protecting groups for peptide synthesis include *t*-butyloxycarbonyl (Boc), fluorenylmethyloxycarbonyl (Fmoc), Benzyl (Bzl), *t*-amyloxycarbonyl (Aoc), tosyl (Tos), benzyloxycarbonyl (Z or Cbz), *o*-bromo-benzyloxycarbonyl (BrZ) and the like. Additional protecting groups are identified in Merrifield, cited above, as well as in McOmie JFW: Protective Groups in Organic Chemistry, Plenum Press, New York, 1973.

[0076] The choice of coupling agent for the method chosen will also be within the skill of a person of ordinary skill in the art. Suitable coupling agents include DCC (N, N'-Dicyclohexylcarbodiimide), Bop (Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate), PyBop (Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate), BopCl (bis(2-oxo-3-oxazolidinyl)phosphinic chloride), 2-Chloro-1,3-dimethylimidazolidinium hexafluorophosphate (CIP) and the like.

[0077] For example, compounds may be synthesized by the following methods.

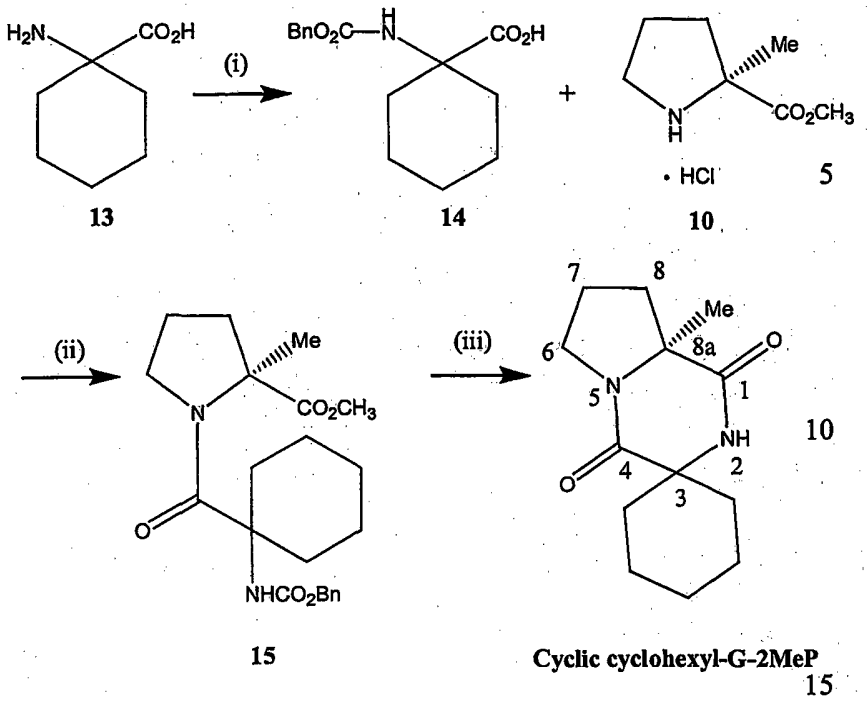


Scheme 1: ***Reagents, conditions and yields:*** (i) LDA, THF, -78 °C, iodomethane, -78 –> -50 °C, 2 h (63%); (ii) $SOCl_2$, CH_3OH, reflux, N_2, 2.5 h (98%); (iii) Et_3N, BoPCl, CH_2Cl_2, RT, N_2, 20.5 h (78%); (iv) 10% Pd/C, CH_3OH, RT, 15 h (98%).

Oxazolidinone 8 can be synthesized by reaction of chloral with proline. This oxazolidinone can then reduced using lithium diisopropylamide (LDA), followed by addition of a methyl group using iodomethane to produce oxazolidinone 9. Thionyl chloride or acetyl chloride can be used to produce the methyl ester of 2-methyl proline as in Scheme 1 above. It will be apparent to those skilled in the art that the iodomethane can be replaced with a suitable halogen compound to produce various analogues modified at the carbon 2 position. For example, use of iodoethane will produce 2-ethyl proline; use of allylbromide will produce 2-allyl proline and use of benzylbromide will produce 2-benzyl proline.

[0078] The proline protected at the C-terminus can then be coupled to an amino acid protected at the N-terminal with a suitable protecting group such as Cbz, Boc or Fmoc. Suitable coupling reagents for this procedure will be apparent to those skilled in the art and include such reagents as bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoPCl), dicyclohexylcarbodiimide (DCC), 2-Chloro-1,3-dimethylimidazolidinium hexafluorophosphate (CIP) (Babu and Ananda, 2001, Indian J. Chem. Sect. B.,: 40B(1): 70; Akaji and Aimoto 2001, Tetrahedron, 57(9), 1749). The dipeptide thus formed can then be selectively de-protected at the N-terminus, using for example, hydrogenation to remove Cbz groups and trifluoroacetic acid (TFA) to remove Boc groups. The molecule then cyclises with elimination of the methoxy group of the methyl ester to give the diketopiperazine.

[0079] The amino acid used in scheme 1 is glycine which gives compounds of formula 1 where R^4=R^5=H. Replacement of glycine with other amino acids will result in compounds of formula 1 where R^4=H and R^5 is equivalent to the side chain of the respective amino acid.



Scheme 2: ***Reagents, conditions and yields:*** **(i) BnO_2CCl, Na_2CO_3, H_2O-dioxane (3:1), 19 h, 96%; (ii) Et_3N, HOAt, CIP, 1,2-dichloroethane, reflux, N_2, 19 h (23%); (iii) 10% Pd/C, CH_3OH, RT, 17 h (65%).**

[0080] 1-Aminocyclohexanecarboxylic acid 13 (Fluka) can be protected at the N-terminus using a protecting group such as Cbz. This compound can then be coupled to a proline derivative suitably protected at the C-terminus using a suitable coupling agent such as 2-Chloro-1,3-dimethylimidazolidinium hexafluorophosphate (CIP) as in Scheme 2. The dipeptide thus formed can then be selectively de-protected at the N-terminus *via* hydrogenation for example, and the resultant elimination of the methoxy group of the methyl ester produces the diketopiperazine. It will be apparent to those skilled in the art that replacement of the 1-aminocyclohexanecarboxylic acid with analogous compounds such as 1-aminocyclopentanecarboxylic acid or 1-aminocyclopropanecarboxylic acid will be possible. It will also be apparent that the methyl group at the C-2 (carbon centre 8a in scheme 2) position of proline may be replaced with other substituents such as ethyl, allyl and benzyl as discussed above.

EXAMPLES

[0081] The present invention is further illustrated by the following examples. These examples are offered by way of illustration only.

General Methods

[0082] Flash chromatography was performed using Scharlau 60 (40-60 μm mesh) silica gel. Analytical thin layer chromatography was carried out on 0.20 mm precoated silica gel plates (ALUGRAM® SIL G/UV_{254}) and compounds visualized using UV fluorescence, or heating of plates dipped in potassium permanganate in alkaline solution.

[0083] Melting points in degrees Celsius (°C) were determined on an Electrothermal® melting point apparatus and are uncorrected.

[0084] Optical rotations were measured at 20 °C on a Perkin Elmer 341 polarimeter using 10 cm path length cells and are given in units of $10^{-1}degcm^2g^{-1}$. Samples were prepared in the solvent indicated at the concentration specified (measured in g/100 cm^3). IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer. The samples

were prepared as thin films on sodium chloride discs or as solids in potassium bromide discs. A broad signal indicated by br. The frequencies (u) as absorption maxima are given in wavenumbers (cm^{-1}).

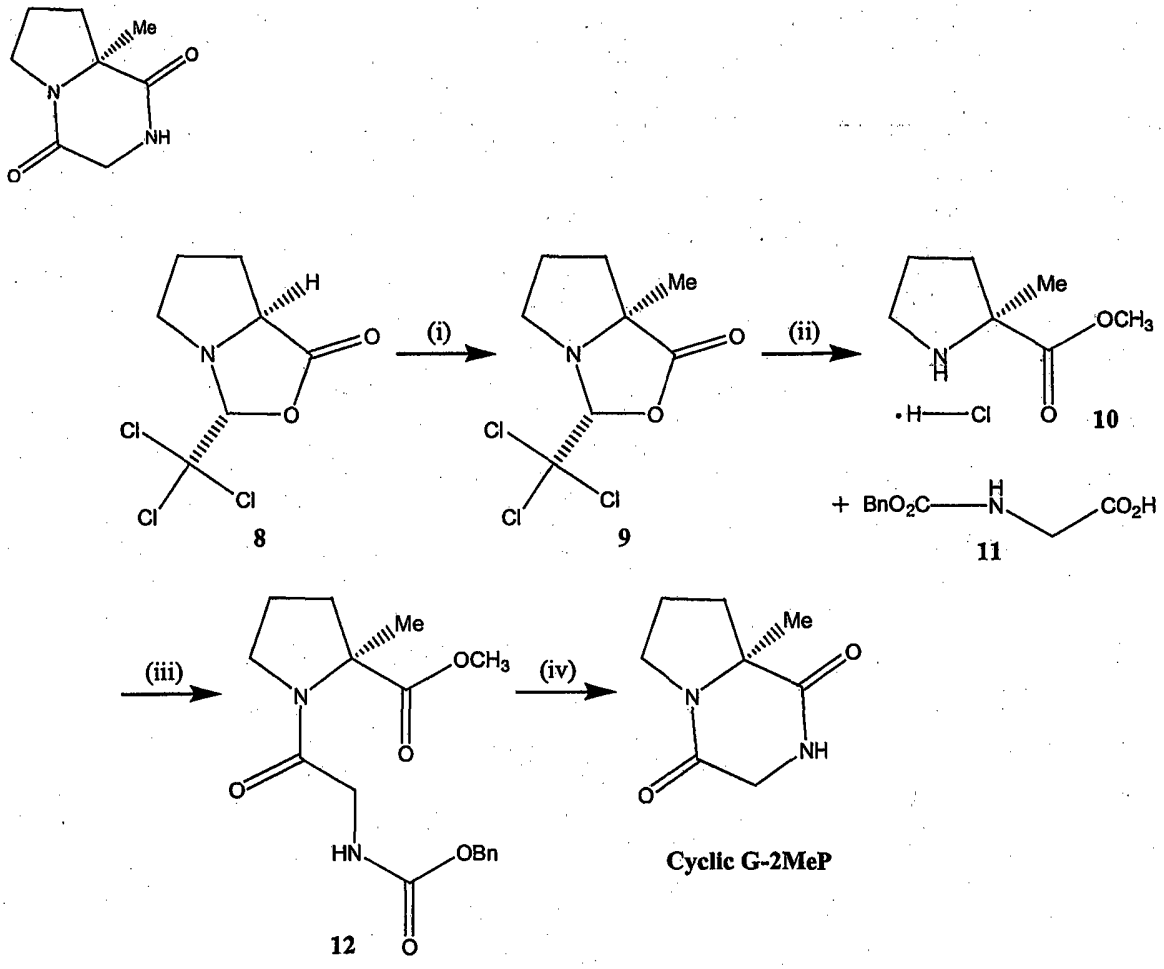
[0085] NMR spectra were recorded on a Bruker AVANCE DRX400 (^1H, 400 MHz; ^{13}C, 100 MHz) or a Bruker AVANCE 300 (^1H, 300 MHz; ^{13}C, 75 MHz) spectrometer at ambient temperatures. For ^1H NMR data chemical shifts are described in parts per million downfield from $SiMe_4$ and are reported consecutively as position (δ_H), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet, br = broad), coupling constant (J/Hz) and assignment. For ^{13}C NMR data, chemical shifts are described in parts per million relative to $CDCl_3$ and are reported consecutively as position (δ_C), degree of hybridization as determined by DEPT experiments, and assignment. ^1H NMR spectra were referenced internally using $SiMe_4$ (δ 0.00) or $CDCl_3$ (δ 7.26). ^{13}C NMR spectra were referenced internally using $CDCl_3$ (δ 77.0). When two sets of peaks arise in the NMR spectra due to different conformations around the glycine-proline amide bond, the chemical shift for the minor *cis* conformer is marked with an asterisk (*).

[0086] Accurate mass measurements were recorded on a VG-70SE mass spectrometer.

[0087] Hexane and dichloromethane were distilled prior to use. Methanol was dried using magnesium turnings and iodine, and distilled under nitrogen. Triethylamine was dried over calcium hydride and distilled under nitrogen.

Example 1: Synthesis of *(8aS)-Methyl-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (Cyclic G-2MeP)*

[0088]



Scheme 1: ***Reagents, conditions and yields:*** (i) LDA, THF, -78 °C, iodomethane, -78 –> -50 °C, 2 h (63%); (ii) $SOCl_2$, CH_3OH, reflux, N_2, 2.5 h (98%); (iii) Et_3N, BoPCl, CH_2Cl_2, RT, N_2, 20.5 h (78%); (iv) 10% Pd/C, CH_3OH, RT, 15 h (98%).

(2R,5S)-4-Methyl-2-trichloromethyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one **9**

[0089] n-BuLi (1.31 M, 4.68 cm^3, 6.14 mmol) was added dropwise to a stirred solution of diisopropylamine (0.86 cm^3, 6.14 mmol) in dry tetrahydrofuran (10 cm^3) at -78 °C under an atmosphere of nitrogen. The solution was stirred for 5 min, warmed to 0 °C and stirred for 15 min. The solution was then added dropwise to a solution of oxazolidinone 8 (1.00 g, 4.09 mmol) in dry tetrahydrofuran (20 cm^3) at -78 °C over 20 min (turned to a dark brown colour), stirred for a further 30 min then iodomethane (0.76 cm^3, 12.3 mmol) was added dropwise over 5 min. The solution was warmed to-50 °C over 2 h. Water (15 cm^3) was added and the solution warmed to room temperature and extracted with chloroform (3 x 40 cm^3). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated to dryness *in vacuo* to give a dark brown semi-solid. Purification of the residue by flash column chromatography (15% ethyl acetate-hexane) afforded *oxazolidinone* **9** (0.67 g, 63%) as a pale yellow solid: mp 55-57 °C (lit., 57-60°C); δ_H (300 MHz), $CDCl_3$) 1.53 (3H, s, CH_3), 1.72-2.02 (3H, m, Proβ-H and Proγ-H_2), 2.18-2.26 (1H, m, Proβ-H), 3.15-3.22 (1H, m, Proδ-H), 3.35-3.44 (1H,m,Proδ-H) and 4.99 (1H, s, NCH).

Methyl L-2-methylprolinate hydrochloride **10**

a) Using acetyl chloride

[0090] Oxazolidinone 9 (0.60 g, 2.33 mmol) was dissolved in dry methanol (15 cm^3) under an atmosphere of nitrogen and acetyl chloride (0.33 cm^3, 4.66 mmol) was added dropwise to the ice-cooled solution. The solution was heated under reflux for 4.5 h, then the solvent removed under reduced pressure to give a brown oil which was purified by flash column chromatography (10% CH_3OH-CH_2Cl_2) affording the *hydrochloride* **10** (0.2 g, 48%) as a flaky white solid: mp 107-109 °C (lit., 106-108 °C); δ_H (300 MHz, $CDCl_3$) 1.81 (3H, s, CH_3), 1.93-2.14 (3H, m, Proβ-H_AH_B and Proγ-H_2), 2.33-2.39 (1H, m, Proβ-H_AH_B), 3.52-3.56 (2H, m, Proδ-H_2) and 3.82 (3H, s, CO_2CH_3).

b) Using thionyl chloride

[0091] An ice-cooled solution of oxazolidinone 9 (53 mg, 0.21 mmol) in dry methanol (1 cm^3) was treated dropwise with thionyl chloride (0.045 cm^3, 0.62 mmol). The solution was heated under reflux for 2.5 h, cooled and the solvent removed under reduced pressure to yield a brown oil. The oil was dissolved in toluene (5 cm^3), concentrated to dryness to remove residual thionyl chloride and methanol then purified by flash column chromatography (10% CH_3OH-CH_2Cl_2) to afford the *hydrochloride* **10** (16 mg, 43%) as a flaky white solid. The ^1H NMR assignments were in agreement with those reported above.

Methyl-N-benzyloxycarbonyl-glycyl-L-2-methylprolinate **12**

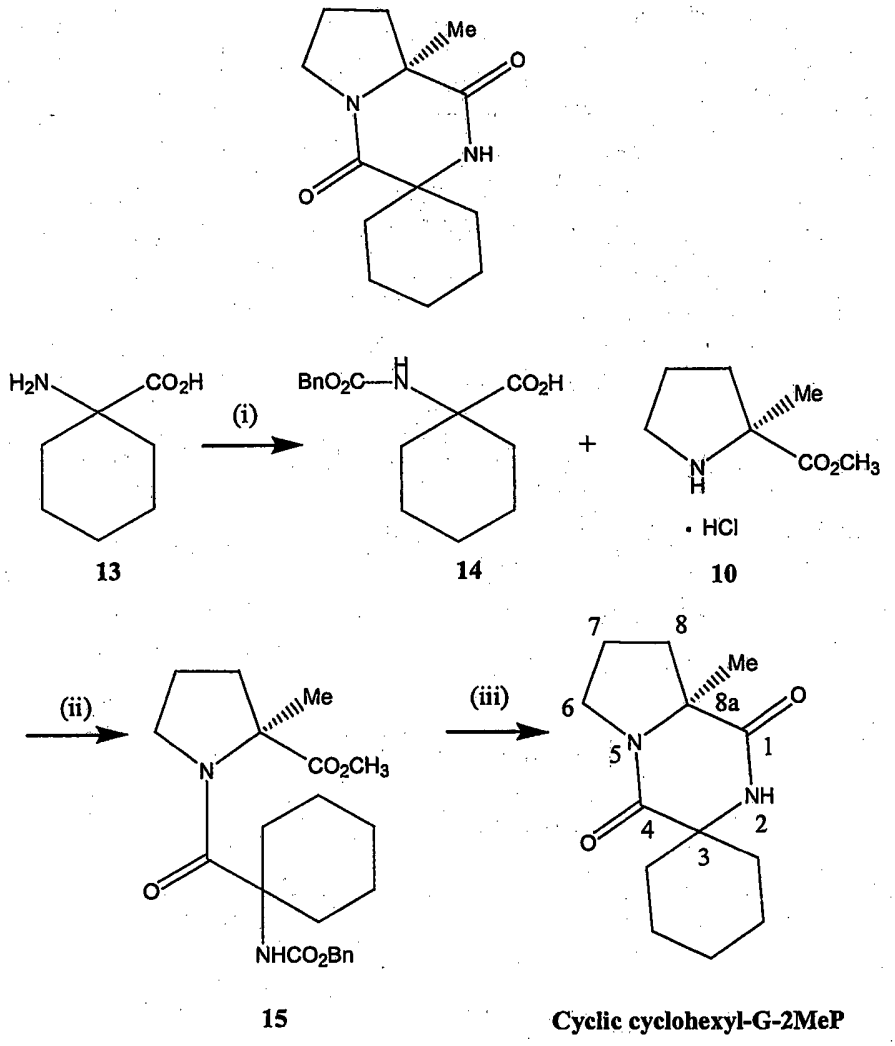
[0092] Dry triethylamine (0.27 cm^3, 1.96 mmol) was added dropwise to a solution of hydrochloride **10** (0.11 g, 0.61 mmol) and *N*-benzyloxycarbonyl-glycine **11** (98.5%) (0.17 g, 0.79 mmol) in dry dichloromethane (35 cm^3) under an atmosphere of nitrogen at room temperature, and the reaction mixture stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoPCl, 97%) (0.196 g, 0.77 mmol) was added and the resultant colourless solution was stirred for 20.5 h. The solution was washed successively with 10% aqueous hydrochloric acid (30 cm^3) and saturated aqueous sodium hydrogen carbonate (30 cm^3), dried ($MgSO_4$), filtered and evaporated to *dryness in vacuo.* Purification of the resultant residue by flash column chromatography (50-80% ethyl acetate-hexane; gradient elution) yielded *dipeptide 12* (0.18 g, 92%) as a colourless oil. Amide **12** was shown to exist as a 98:2 *trans:cis* mixture of conformers by ^{13}C NMR analysis (the ratio was estimated from the relative intensities of the resonances at δ 20.8 and 23.5 assigned to the Proy-C atoms of the minor and major conformers, respectively): $[\alpha]_D$ -33.0 (c 1.0 in MeOH); ν_{max} (film)/cm^{-1} 3406, 2952, 1732, 1651, 1521, 1434, 1373, 1329, 1310, 1284, 1257, 1220, 1195, 1172, 1135, 1107, 1082, 1052, 1029, 986, 965, 907, 876, 829, 775, 738 and 699; δ_H (300 MHz, $CDCl_3$) 1.49 (3H, s, CH_3), 1.77-2.11 (4H, m, Proβ-H_2 and Proγ-H_2), 3.43-3.48 (2H, m, Proδ-H_2), 3.61 (3H, s, OCH_3), 3.85-3.89 (2H, m, Glyα-H_2), 5.04 (2H, s, PhCH_2), 5.76 (1H, br s, N-H) and 7.21-7.28 (5H, s, ArH); δ_C (75 MHz, $CDCl_3$) 13.8* (CH_3, Proα-CH_3), 21.1 (CH_3, Proα-CH_3), 20.8* (CH_2, Proγ-C), 23.5 (CH_2, Proγ-C), 38.0 (CH_2, Proβ-C), 40.8* (CH_2, Proβ-C), 43.3 (CH2, Glyα-C), 45.5* (CH_2, Glyα-C), 46.6 (CH_2, Proδ-C), 48.7* (CH_2, Proδ-C), 51.9* (CH_3, OCH_3), 52.1 (CH3, OCH_3), 60.0* (quat., Proα-C), 66.0 (quat., Proα-C), 66.3 (CH_2, PhCH_2), 68.6* (CH_2, PhCH_2), 127.5 (CH, Ph), 127.6 (CH, Ph), 127.9* (CH, Ph), 128.1 (CH, Ph), 128.3* (CH, Ph), 136.2 (quat., Ph), 155.9 (quat., NCO_2), 166.0 (quat., Gly-CON), 169.4* (quat., Gly-CON) and 173.6 (quat., CO_2CH_3); *m/z* (EI+) 334.1535 (M^+. $C_{17}H_{22}N_2O_5$ requires 334.1529).

*(8aS)-Methyl-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (Cyclic **G-2MeP**)*

[0093] To a solution of dipeptide **12** (0.167 g, 0.51 mmol) in methanol (8.0 cm^3) was added 10% Pd on activated charcoal (8.1 mg, 0.076 mmol) and the vessel flushed with hydrogen gas. The resulting suspension was stirred vigorously under an atmosphere of hydrogen for 15 h. The mixture was then filtered through a Celite pad then a short plug of silica gel with methanol, and the solvent removed under reduced pressure to produce ***cyclic G-2MeP*** **(83 mg,** 98%) as a yellow solid: mp 133-135 °C; $[\alpha]_D$ -128.1 (c 0.52 in MeOR); (300 MHz, $CDCl_3$) 1.36 (3H, s, CH_3); 1.87-2.01 (3H, m, Proβ-H_AH_B and Proγ-H_2), 2.07-2.21 (1H, m, Proβ-H_AH_B), 3.45-3.64 (2H, m, Proδ-H_2), 3.82 (1H, dd, *J* 17.1 and 4.1, CH_AH_BNH), 3.99 (1H, d, *J* 17.1, CH_AH_BNH) and 7.66 (1H, br s, N-H); δ_C (75 MHz, $CDCl_3$) 20.2 (CH_2, Proγ-C), 23.2 (CH_3, Proα-CH_3), 35.0 (CH_2, Proβ-C), 44.7 (CH_2, Proδ-C), 45.9 (CH_2, CH_2NH), 63.8 (quat., Proα-C), 163.3 (quat., NCO) and 173.3 (quat., CONH); *m/z* (EI+) 168.08986 (M^+. $C_8H_{12}N_2O_2$ requires 168.08988).

Example 2: Synthesis of *(8aS)-Methyl-spiro[cyclohexane-1,3(4H)-tetrahydropyrrolo[1,2-a]pyrazine]-1,4(2H)-dione (Cyclic cyclohexyl-G-2MeP)*

[0094]



Scheme 2: ***Reagents, conditions and yields:*** (i) BnO_2CCl, Na_2CO_3, H_2O-dioxane (3:1), 19 h, 96%; (ii) Et_3N, HOAt, CIP, 1,2-dichloroethane, reflux, N_2, 19 h (23%); (iii) 10% Pd/C, CH_3OH, RT, 17 h (65%).

N-benzyloxycarbonyl-1-aminocyclohexane-1-carboxylic acid (14)

[0095] To a suspension of 1-aminocyclohexanecarboxylic acid 13 (0.72 g, 5.02 mmol) and sodium carbonate (1.6 g, 15.1 mmol) were dissolved in water-dioxane (21 cm^3, 3:1) was added benzyl chloroformate (0.79 cm^3, 5.52 mmol) was added dropwise and the solution was stirred at room temperature for 19.5 h. The aqueous layer was washed with diethyl ether (60 cm^3), acidified with 2 M HCl and extracted with ethyl acetate (2 x 60 cm^3). The organic layers were combined, dried ($MgSO_4$), filtered and evaporated under reduced pressure to produce a colourless oil, which solidified on standing to crude *carbamate* **14** (1.23 g, 88%) as a white solid: mp 152-154 °C (lit, 148-150°C); δ_H (400 MHz, $CDCl_3$) 1.27-1.56 (3H, m, 3 x cyclohexyl-H), 1.59-1.73 (3H, m, 3 x cyclohexyl-H), 1.85-1.91 (2H, m, 2 x cyclopentyl-H), 2.05-2.09 (2H, m, 2 x cyclopentyl-H), 5.02 (1H, br s, N-H), 5.12 (2H, s, OCH_2Ph) and 7.27-7.36 (5H, s, Ph); δ_C (100 MHz, $CDCl_3$) 21.1 (CH_2, 2 x cyclohexyl-C), 25.1 (CH_2, 2 x cyclohexyl-C), 32.3 (CH_2, cyclohexyl-C), 59.0 (quat.,1-C), 67.1 (CH_2, OCH_2Ph), 128.1 (CH, Ph), 128.2 (CH, Ph), 128.5 (CH, Ph), 136.1 (quat., Ph), 155.7 (quat., NCO_2) and 178.7 (quat., CO_2H).

Methyl-N-benzyloxycarbonyl-cyclohexyl-glycyl-L-2-methylprolinate (**15**)

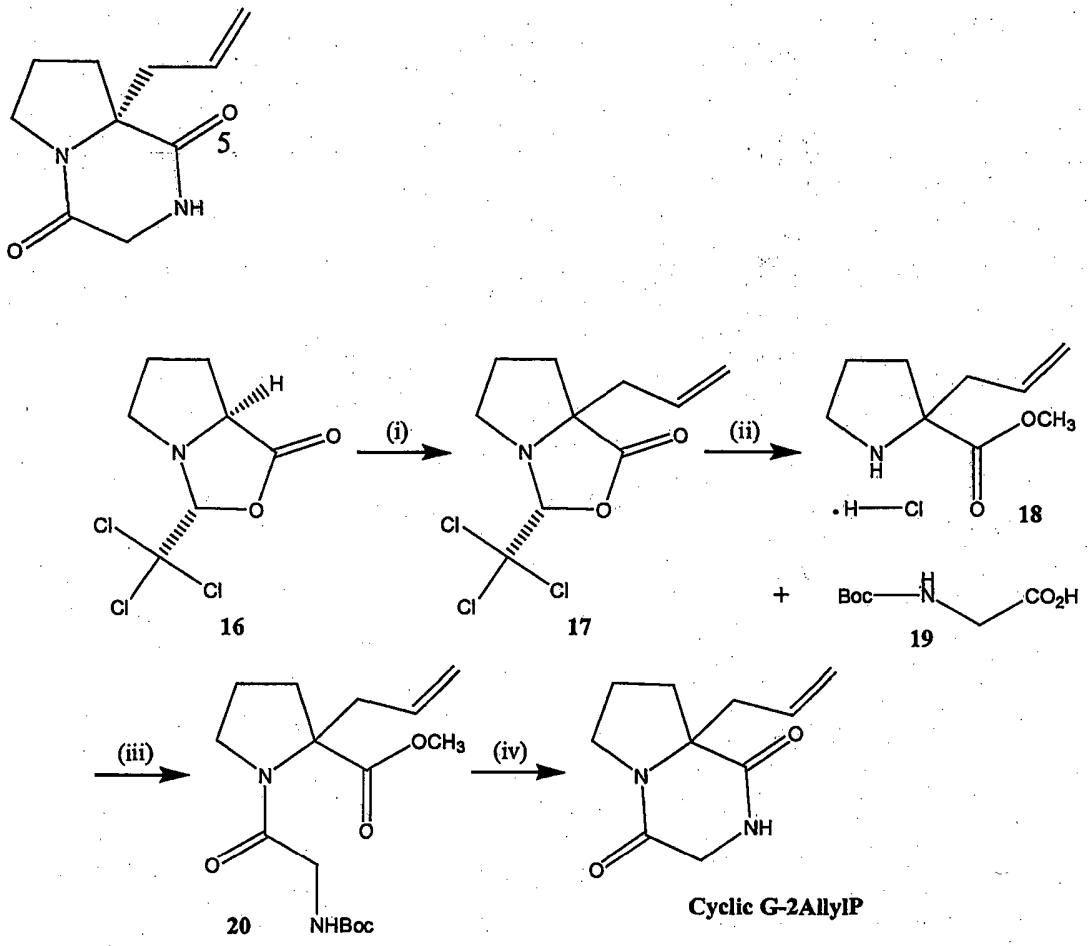
[0096] Dry triethylamine (0.21 cm^3, 1.5 mmol) was added dropwise to a solution of hydrochloride **10** (84.0 mg, 0.47 mmol), carboxylic acid **14** (0.17 g, 0.61 mmol) and 1-hydroxy-7-azabenzotriazole (16 mg, 0.12 mmol) in dry 1,2-dichloroethane (26 cm^3) under an atmosphere of nitrogen at room temperature, and the reaction mixture stirred for 10 min. 2-Chloro-1,3-dimethylimidazolidinium hexafluorophosphate (0.13 g, 0.47 mmol) was added and the resultant solution heated under reflux for 21 h, then washed successively with 10% aqueous hydrochloric acid (30 cm^3) and saturated aqueous sodium hydrogen carbonate (30 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness *in vacuo.* Purification of the resultant residue by flash column chromatography (40-50% ethyl acetate-hexane; gradient elution) yielded *amide* **15** (16 mg, 9%) as a white solid. Amide **15** was shown to exist as a 11:1 *trans:cis* mixture of conformers by ^{13}C NMR analysis (the ratio was estimated from the relative intensities of the resonances at δ 41.3 and 48.2 assigned to the Proδ-C atoms of the minor and major conformers, respectively): mp 219-222 °C; $[\alpha]_D$ -44.9 (*c* 1.31 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3239, 2927, 1736, 1707, 1617, 1530, 1450, 1403, 1371, 1281, 1241, 1208, 1194, 1165, 1150, 1132, 1089, 1071, 1028, 984, 912, 796, 749, 739 and 699; δ_H (400 MHz, $CDCl_3$) 1.24-2.10 (17H, m, Proα-CH_3, Proβ-H_2, Proγ-H_2 and 5 x cyclohexyl-H_2), 3.25-3.48 (1H, br m, Proδ-H_AH_B), 3.61-3.87 (4H, br m, OCH_3 and Proδ-H_AH_B), 4.92-5.19 (3H, m, N-H and OCH_2Ph) and 7.35-7.37 (5H, s, Ph); δ_C (100 MHz, $CDCl_3$) 21.26 (CH_2, cyclohexyl-C), 21.33 (CH_2, cyclohexyl-C), 21.7 (CH_3, Proα-CH_3), 24.8 (CH_2, cyclohexyl-C), 25.0 (CH_2, Proγ-C), 29.4* (CH_2, cyclohexyl-C), 29.7* (CH_2, cyclohexyl-C), 31.1 (CH_2, cyclohexyl-C), 31.6 (CH_2, cyclohexyl-C), 31.9* (CH_2, cyclohexyl-C), 32.2* (CH_2, cyclohexyl-C); 32.8* (CH_2, cyclohexyl-C), 37.3 (CH_2, Proβ-C), 41.4* (CH_2, Proδ-C), 48.2 (CH_2, Proδ-C), 52.1 (CH_3, OCH_3), 59.1 (quat, Glyα-C), 66.7 (CH_2, OCH_2Ph), 67.3* (CH_2, OCH_2Ph), 67.4 (quat., Proα-C), 128.0* (CH, Ph), 128.1* (CH, Ph), 128.3 (CH, Ph), 128.5 (CH, Ph), 128.7 (CH, Ph), 136.6 (quat., Ph), 153.7 (quat., NCO_2), 171.0 (quat., Gly-CO) and 174.8 (quat., CO_2CH_3); *m/z* (EI+) 402.2151 (M^+. $C_{22}H_{30}N_2O_5$ requires 402.2155).

(8aS) Methyl-spiro[cyclohexane-1,3(4H)-tetrahydropyrrolo[1,2-a]pyrazine]-1,4(2H)-dione ***(Cyclic cyclohexyl-G-2MeP)***

[0097] To a solution of amide **15** (40 mg, 0.01 mmol) in methanol (3.3 cm^3) was added 10% Pd on activated charcoal (1.6 mg, 0.015 mmol) and the vessel flushed with hydrogen gas. The resulting suspension was stirred vigorously under an atmosphere of hydrogen for 61.5 h, then filtered through a Celite™ pad with methanol (15 cm^3). The filtrate was concentrated to dryness under reduced pressure to produce a yellow semi-solid which was purified by reverse-phase C18 flash column chromatography (0-10% CH_3CN/H_2O; gradient elution) to produce ***cyclic cyclohexyl G-2MeP*** (19 mg, 81%) as a white solid: mp 174-177 °C; $[\alpha]_D$ -63.8 (c 1.13 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3215, 2925, 2854, 1667, 1646, 1463, 1427, 1276, 1232, 1171, 1085, 1014, 900, 868, 818, 783, 726 and 715; δ_H (400 MHz, $CDCl_3$) 1.31-1.89 (12H, m, 9 x cyclohexyl-H and 8a-CH_3), 1.94-2.15 (4H, m, 7-H_2 and 8-H_2), 2.26(1H, td, *J* 13.7 and 4.5, 1 x cyclohexyl-H), 3.44-3.51 (1H, m, 6-H_AH_B), 3.79-3.86 (1H, m, 6-H_AH_B) and 6.40 (1H, br s, N-H); δ_C (100 MHz, $CDCl_3$) 19.5 (CH_2, 7-C), 20.6 (CH_2, cyclohexyl-C), 20.8 (CH_2, cyclohexyl-C), 24.5 (CH_2, cyclohexyl-C), 25.0 (CH_3, 8a-CH_3), 33.7 (CH_2, cyclohexyl-C), 36.3 (CH_2, 8-C), 36.5 (CH_2, cyclohexyl-C), 44.7 (CH_2, 6-C), 59.5 (quat., 8a-C), 64.0 (quat., 3-C), 168.1 (quat., 4-C) and 171.6 (quat., 1-C); *m/z* (EI+) 236.15246 (M^+. $C_{13}H_{20}N_2O_2$ requires 236.15248).

Example 3: Synthesis of ***(8aS)-Allyl-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (Cyclic G-2AllylP)***

[0098]



Scheme 3: ***Reagents, conditions and yields:*** **(i) LDA, THF, -78 °C, allyl bromide, -78 –> -30 °C, N_2, 4 h (60%); (ii) acetyl chloride, CH_3OH, reflux, N_2, 24 h (63%); (iii) Et_3N, BoPCl, CH_2Cl_2, RT, N_2, 19.5 h (45%); (iv) TFA, CH_2Cl_2, 1 h, then Et_3N, CH_2Cl_2, 23 h (37%).**

(2R,5S)-4-Allyl-2-trichloromethyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one **17**

[0099] n-BuLi (1.31 M, 9.93 cm^3, 13.0 mmol) was added dropwise to a stirred solution of diisopropylamine (1.82 cm^3, 13.0 mmol) in dry tetrahydrofuran (20 cm^3) at -78 °C under an atmosphere of nitrogen. The solution was stirred for 5 min, warmed to 0 °C, stirred for 15 min then added dropwise to a solution of pro-oxazolidinone 16 (2.12 g, 8.68 mmol) in dry tetrahydrofuran (40 cm^3) at -78 °C over 20 min and the reaction mixture was stirred for a further 30 min then allyl bromide (2.25 cm^3, 26.0 mmol) was added dropwise over 5 min. The solution was warmed slowly to -30 °C over 4 h, quenched with H_2O (30 cm^3) and the mixture warmed to room temperature and extracted with chloroform (3 x 80 cm^3). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated to dryness *in vacuo* to produce a dark brown semi-solid which was purified by flash column chromatography (10-20% ethyl acetate-hexane; gradient elution) to produce *oxazolidinone* 17 (1.48 g, 60%) as an orange oil which solidified at 0 °C, for which the nmr data were in agreement with that reported in the literature: δ_H (400 MHz, $CDCl_3$) 1.58-1.92 (2H, m, Proγ-H_2), 1.96-2.14 (2H, m, Proβ-H_2), 2.50-2.63 (2H, m, Proδ-H_2), 3.12-3.23 (2H, m, CH_2-CH=CH_2), 4.97 (1H, s, NCH), 5.13-5.18 (2H, m, CH=CH_2) and 5.82-5.92 (1H, m, C*H*=CH_2); δ_C (100 MHz, $CDCl_3$) 25.1 (CH_2, Proγ-C), 35.1 (CH_2, Proβ-C), 41.5 (CH_2, Proδ-C), 58.3 (CH_2. $CH_2CH=CH_2$), 71.2 (quat., Proα-C), 100.4 (quat., CCl_3), 102.3 (CH, NCH), 119.8 (CH_2, $CH_2CH=CH_2$), 131.9 (CH, $CH_2CH=CH_2$) and 176.1 (quat., C=O); *m/z* (CI+) 284.0009 [$(M+H)^+$. $C_{10}H_{13}{}^{35}Cl_3NO_2$ requires 284.0012], 285.9980 [$(M+H)^+$. $C_{10}H_{13}{}^{35}Cl_2{}^{37}ClNO_2$ requires 285.9982], 287.9951 [$(M+H)^+$. $C_{10}H_{13}{}^{35}Cl^{37}Cl_2NO_2$ requires 287.9953] and

289.9932 [(M+H)$^+$. $C_{10}H_{13}{}^{37}Cl_3NO_2$ requires 289.9923].

Methyl L-2-allylprolinate hydrochloride ***18***

[0100] An ice-cooled solution of oxazolidinone **17** (0.64 g, 2.24 mmol) in dry methanol (15 cm^3) was treated dropwise with a solution of acetyl chloride-(0.36 cm^3, 5.0 mmol) in methanol (5 cm^3). The solution was heated under reflux for 24 h, then cooled and the solvent removed under reduced pressure. The resultant brown oil was dissolved in toluene (40 cm^3) and concentrated to dryness to remove residual thionyl chloride and methanol, then purified by flash column chromatography (5-10% CH_3OH-CH_2Cl_2; gradient elution) to afford *hydrochloride* **18** (0.29 g, 63%) as a green solid for which the NMR data were in agreement with that reported in the literature: δ_H (300 MHz, $CDCl_3$) 1.72-2.25 (3H, m, Proβ-H_AH_B and Proγ-H_2), 2.32-2.52 (1H, m, Proβ-H_AH_B), 2.72-3.10 (2H, m, Proδ-H_2), 3.31-3.78 (2H, m, $CH_2CH{=}CH_2$), 3.84 (3H, s, CO_2CH_3), 5.20-5.33 (2H, m, CH=CH_2), 5.75-5.98 (1H, m, $CH{=}CH_2$) and 8.06 (1H, br s, N-H); *m/z* (CI+) 170.1183 [(M+H)$^+$. $C_9H_{16}NO_2$ requires 170.1181].

Methyl-N-tert-butyloxycarbonyl-glycyl-L-2-allylprolinate **20**

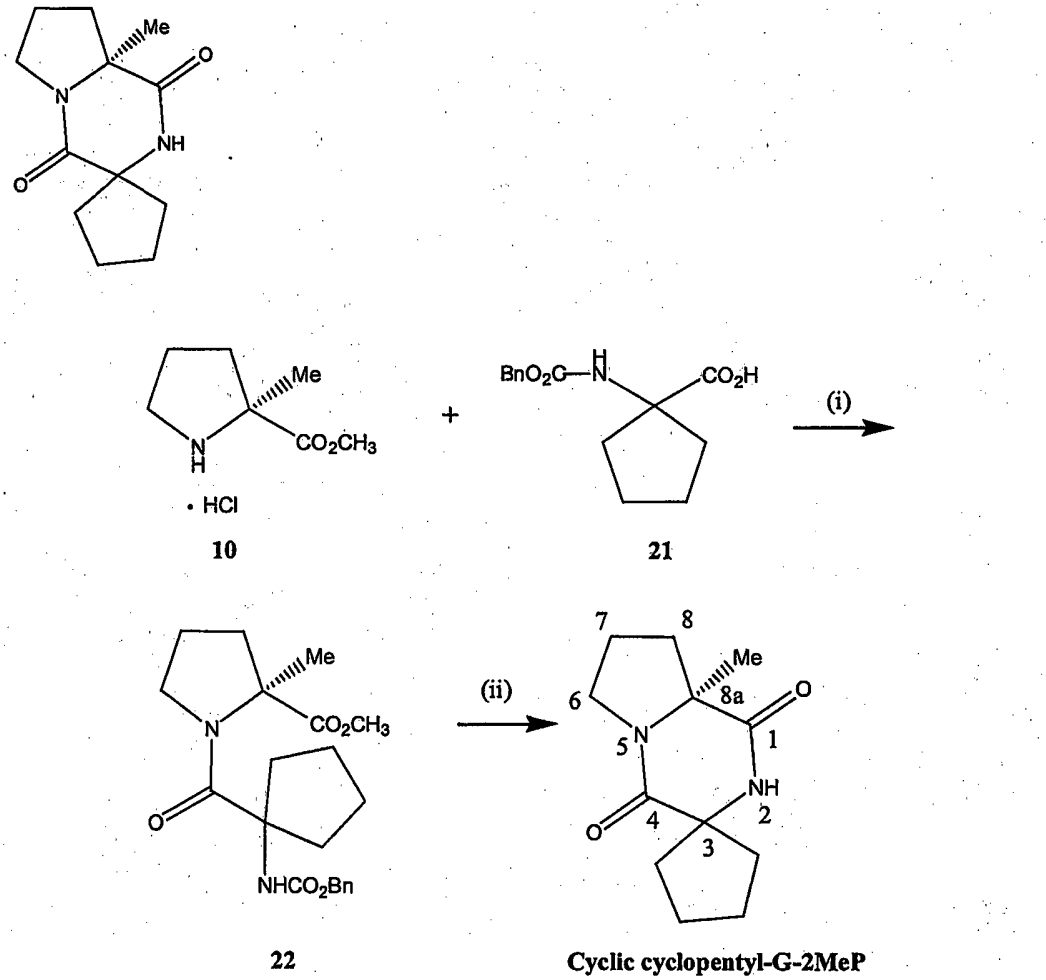
[0101] Dry triethylamine (0.28 cm^3, 2.02 mmol) was added dropwise to a solution of hydrochloride **18** (0.13 g, 0.63 mmol) and *N-tert*-butyloxycarbonyl-glycine **19** (0.14 g, 0.82 mmol) in dry dichloromethane (35 cm^3) under an atmosphere of nitrogen at room temperature, and the reaction mixture was stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoPCl, 97%) (0.20 g, 0.80 mmol) was added and the solution stirred for 19.5 h, then washed successively with 10% aqueous hydrochloric acid (35 cm^3) and saturated aqueous sodium hydrogen carbonate (35 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness in *vacuo*. Purification of the residue by flash column chromatography (40% ethyl acetate-hexane) yielded *dipeptide* **20** (0.09 g, 45%) as a light yellow oil: $[\alpha]_D$ +33.8 (c 0.83 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3419, 3075, 2977, 2930, 2874, 1739, 1715, 1656, 1499, 1434, 1392, 1366, 1332, 1268, 1248, 1212, 1168, 1122, 1051, 1026, 1003, 943, 919, 867, 830, 779, 739, 699 and 679; δ_H (300 MHz, $CDCl_3$) 1.42 [9H, s, $C(CH_3)_3$], 1.93-2.08 (4H, m, Proβ-H_2 and Proγ-H_2), 2.59-2.67 (1H, m, $CH_AH_BCH{=}CH_2$), 3.09-3.16 (1H, m, $CH_AH_BCH{=}CH_2$), 3.35-3.44 (1H, m, Proδ-H_AH_B), 3.56-3.62 (1H, m, Proδ-H_AH_B), 3.70 (3H, s, OCH_3), 3.89 (2H, d, *J* 4.2, Glyα-H_2), 5.06-5.11 (2H, m, CH=CH_2), 5.42 (1H, br s, Gly-NH) and 5.58-5.72 (1H, m, $CH{=}CH_2$); δ_C(75 MHz, $CDCl_3$) 23.7 (CH_2, Proγ-C), 28.3 [CH_3, $C(CH_3)_3$], 35.0 (CH_2, Proβ-C), 37.6 (CH_2, $CH_2CH{=}CH_2$), 43.3 (CH_2, Glyα-C), 47.5 (CH_2, Proδ-C), 52.5 (CH_3, OCH_3), 68.8 (quat., Proα-C), 79.5 [quat., $C(CH_3)_3$], 119.4 (CH_2, CH=CH_2), 132.9 (CH, $CH{=}CH_2$), 155.7 (quat., NCO_2), 166.9 (quat., Gly-CON) and 173.8 (quat., CO_2CH_3); *m/z* (EI+) 326.1845 (M$^+$. $C_{16}H_{26}N_2O_5$ requires 326.1842).

*(8aS)-Allyl-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (****Cyclic G-2AllylP****)*

[0102] To a solution of dipeptide **20** (0.09 g, 0.28 mmol) in dichloromethane (9 cm^3) at room temperature was added trifluoroacetic acid (1 cm^3, 0.013 mmol) dropwise and the reaction mixture was stirred for 1 h under an atmosphere of nitrogen. The solution was evaporated under reduced pressure to give a colorless oil which was dissolved in dichloromethane (10 cm^3), dry triethylamine (0.096 cm^3, 0.69 mmol) was added and the reaction mixture stirred for 4.5 h, after which further triethylamine (0.096 cm^3, 0.69 mmol) was added. The reaction mixture was stirred overnight, concentrated to dryness to give a green oil which was purified by flash column chromatography (10% CH_3OH-CH_2Cl_2) to produce ***cyclic G-2AllylP*** (20 mg, 37%) as an off-white solid: mp 106-109 °C; $[\alpha]_D$ -102.7 (c 0.95 in CH_2Cl_2); ν_{max} (CH_2Cl_2)/cm^{-1} 3456, 3226, 2920, 1666, 1454, 1325, 1306, 1299, 1210, 1133, 1109, 1028, 1010, 949, 928, 882, 793, 761 and 733; δ_H (400 MHz, $CDCl_3$) 1.92-2.01 (2H, m, Proγ-H_2), 2.09-2.16 (2H, m, Prop-H_2), 2.39-2.56 (2H, m, $CH_2CH_2{=}CH_2$), 3.46-3.53 (1H, m, Proδ-H_AH_B), 3.78-3.87 (2H, m, Proδ-H_AH_B and Glyα-H_AH_B), 4.09 (1H, d, *J* 17.2, Glyα-H_AH_B), 5.16-5.20 (2H, m, CH=CH_2), 5.73-5.84 (1H, m, $CH{=}CH_2$) and 7.17 (1H, br s, N-H); δ_C (100 MHz, $CDCl_3$) 20.1 (CH_2, Proγ-C), 34.1 (CH_2, Proβ-C), 41.7 (CH_2, $CH_2CH_2{=}CH_2$), 44.9 (CH_2, Proδ-C), 46.4 (CH_2, Glyα-C), 67.2 (quat., Proα-C), 120.9 (CH_2, CH=CH_2), 131.0 (CH, $CH{=}CH_2$), 163.4 (quat., NCO) and 171.7 (quat., CONH); *m/z* (EI+) 195.1132 (M$^+$. $C_{10}H_{15}N_2O_2$ requires 195.1134).

Example 4: Synthesis of *(8aS)-Methyl-spiro[cyclopentane-1,3(4H)-tetrahydropyrrolo[1,2-a]pyrazine]-1,4(2H)-dione (Cyclic Cyclopentyl-G-2MeP)*

[0103]



Scheme 4: ***Reagents, conditions and yields:*** (i) Et_3N, HOAt, CIP, 1,2-dichloroethane, 83 °C, N_2, 19 h (23%); (ii) 10% Pd/C, CH_3OH, RT, 17 h (65%).

N-Benzyloxycarbonyl-1-aminocyclopentane-1-carboxylic acid **21**

[0104] A solution of benzyl chloroformate (0.290 g, 1.1 mmol) in dioxane (2.5 cm^3) was added dropwise to a solution of 1-aminocyclopentanecarboxylic acid (Fluka) (0.2 g, 1.54 mmol) and sodium carbonate (0.490 g, 4.64 mmol) in water (5 cm^3) at 0 °C. Stirring was continued at room temperature overnight and the reaction mixture washed with ether. The aqueous layer was acidified with 2M hydrochloric acid, extracted with ethyl acetate, dried (Na_2SO_4), filtered and the solvent removed to afford *carbamate* **21** (0.253 g, 62%) as an oil which solidified on standing. Carbamate **21** was shown to be a 70:30 mixture of conformers by ^1H NMR analysis (the ratio was estimated from the integration of the resonances at δ 5.31 and 7.29-7.40, assigned to the N-H protons of the major and minor conformers, respectively): mp 70-80 °C (lit.[1] 82-86 °C, ethyl acetate, petroleum ether); δ_H (400 MHz; $CDCl_3$; Me4Si) 1.83 (4H, br s, 2 x cyclopentyl-H_2), 2.04 (2H, br s, cyclopentyl-H_2), 2.20-2.40 (2H, m, cyclopentyl-H_2), 5.13 (2H, br s, OCH_2Ph), 5.31 (0.7H, br s, N-H) and 7.29-7.40 (5.3H, m, Ph and N-H*); δ_C (100 MHz; $CDCl_3$) 24.6 (CH_2, cyclopentyl-C), 37.5 (CH_2, cyclopentyl-C), 66.0 (quat., cyclopentyl-C), 66.8 (CH_2, OCH_2Ph), 128.0 (CH, Ph), 128.1 (CH, Ph), 128.4 (CH, Ph), 136.1 (quat, Ph), 155.8 (quat., NCO_2) and 179.5 (quat., CO_2H).

Methyl N-benzyloxycarbonyl cyclopentyl-glycyl-L-2-methylprolinate **22**

[0105] Dry triethylamine (0.19 cm^3, 1.4 mmol) was added dropwise to a solution of hydrochloride **10** (78 mg, 0.43 mmol), carboxylic acid **21** (0.15 g, 0.56 mmol) and 1-hydroxy-7-azabenzotriazole (Acros) (15 mg, 0.11 mmol) in dry 1,2-dichloroethane (24 cm^3) under an atmosphere of nitrogen at room temperature, and the reaction mixture stirred for 10

min. 2-Chloro-1,3-dimethylimidazolidinium hexafluorophosphate (CIP) (Aldrich) (0.12 g, 0.43 mmol) was added and the resultant solution heated under reflux for 19 h, then washed successively with 10% aqueous hydrochloric acid (30 cm^3) and saturated aqueous sodium hydrogen carbonate (30 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness *in vacuo.* Purification of the resultant residue by flash column chromatography (60% ethyl acetate-hexane) yielded *amide* **22** (39 mg, 23%) as a white solid. Amide **22** was shown to exist as a 3:1 *trans: cis* mixture of carbamate conformers by ^{13}C NMR analysis (the ratio was estimated from the relative intensities of the resonances at δ 154.1 and 155.7 assigned to the carbamate carbonyl-C atoms of the major and minor conformers, respectively): mp 200-203 °C; $[\alpha]_D$ -54.5 (c 1.52 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3432, 3239, 3042, 2953, 1736, 1712, 1627, 1540, 1455, 1417, 1439, 1374, 1282, 1256, 1216, 1194, 1171, 1156, 1136, 1100, 1081, 1042, 1020, 107, 953, 917, 876, 756 and 701; δ_H (400 MHz, $CDCl_3$) 1.33-1.53 (3H, br m, Proα-CH_3), 1.62-2.20 (11H, m, Proβ-H_2, Proγ-H_2 and 7 x cyclopentyl-H), 2.59-2.71 (1H, br m, 1 x cyclopentyl-H), 3.31-3.42 (1H, br m, Proδ-H_AH_B), 3.58-3.79 (4H, br m, OCH_3 and Proδ-H_AH_B), 4.92-5.17 (3H, m, N-H and OCH_2Ph) and 7.27-7.42 (5H, s, Ph); δ_C (100 MHz, $CDCl_3$) 21.7 (CH_3, Proα-CH_3), 24.1* (CH2, cyclopentyl-C), 24.2 (CH_2, cyclopentyl-C), 24.4 (CH_2, Proγ-C), 24.5 (CH_2, cyclopentyl-C), 36.4 (CH_2, cyclopentyl-C), 37.1 (CH_2, cyclopentyl-C), 37.2* (CH_2, cyclopentyl-C), 37.7 (CH_2, Proβ-C), 38.2* (CH_2, cyclopentyl-C), 48.5 (CH_2, Proδ-C), 52.1 (CH_3, OCH_3), 66.6 (CH_2, OCH_2Ph), 66.9 (quat., Proα-C), 67.2 (quat., Glyα-C), 127.8 (CH, Ph), 128.2 (CH, Ph), 128.4 (CH, Ph), 136.6 (quat., Ph), 154.1 (quat., NCO_2), 155.7* (quat., NCO_2), 170.5 (quat., Gly-CO) and 174.7 (quat., CO_2CH_3); *m/z* (EI+) 388.1991 (M^+. $C_{21}H_{28}N_2O_5$ requires 388.1998).
* denotes resonance assigned to minor conformer.

(8aS)-Methyl-spiro[cyclopentane-1,3(4H)-tetrahydropyrrolo[1,2-a]pyrazine]-1,4(2H)-dione ***(Cyclic cyclopentyl-G-2MeP)***

[0106] To a solution of amide **22** (54 mg, 0.14 mmol) in methanol (4.6 cm^3) was added 10% Pd on activated charcoal (2.2 mg, 0.021 mmol) and the vessel flushed with hydrogen gas. The resulting suspension was stirred vigorously under an atmosphere of hydrogen for 17 h, then filtered through a Celite™ pad with methanol (15 cm^3). The filtrate was concentrated to dryness under reduced pressure to give a yellow semi-solid which was purified by reverse-phase C18 flash column chromatography (0-10% CH_3CN/H_2O; gradient elution) to afford ***cyclic cyclopentyl-G-2MeP*** (20 mg, 65%) as a yellow solid: mp 160-163 °C; $[\alpha]_D$ -97.9 (c 1.61 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3429, 2956, 2928, 2856, 1667, 1643, 1463, 1432, 1373, 1339, 1254, 1224, 1175, 1086, 1048, 976, 835, 774 and 730; δ_H (300 MHz, $CDCl_3$) 1.47 (3H, br s, 8a-CH_3), 1.56-2.19 (11H, m, 8-H_2, 7-H_2 and 7 x cyclopentyl), 2.58-2.67 (1H, br m, 1 x cyclopentyl), 3.48-3.56 (1H, m, 6-H_AH_B), 3.72-3.82 (1H, m, 6-H_AH_B) and 6.56 (1H, br s, N-H); δ_C (75 MHz, $CDCl_3$) 19.9 (CH_2, 7-C), 24.6 (CH_2, cyclopentyl), 24.92 (CH_3, 8a-CH_3), 24.93 (CH_2, cyclopentyl), 36.0 (CH_2, 8-C), 38.7 (CH_2, cyclopentyl), 41.9 (CH_2, cyclopentyl), 44.8 (CH_2, 6-C), 64.3 (quat., 8a-C), 66.8 (quat., 3-C), 168.3 (quat., 4-C) and 172.2 (quat., 1-C); *m/z* (EI+) 222.1369 (M^+. $C_{12}H_{18}N_2O_2$ requires 222.1368).

In Vitro and *In Vivo* Testing

[0107] The following pharmacological studies demonstrate neuroprotective features of the compouns of this invention. All the following experiments were carried out using protocols developed under guidelines approved by the University of Auckland Animal Ethics Committee.

Example 5: Effects of Cyclic G-2AllylP and Cyclic cyclopentyl-G-2MeP on Cerebellar Cell Explants

[0108] To determine the effects of cG-2AllylP and cyclic cyclopentyl-G-2MeP on neuronal cells *in vitro,* a series of studies was carried out using cerebellar explants from adult rats. *In vitro* systems are suitable for studying neuronal proliferation, neurite growth, formation of nerve bundles and effects of toxins on neural cells, effects that parallel effects observed *in vivo.* Thus, results of studies using *in vitro* cerebellar explants are predictive of effects of interventions *in vivo.*

[0109] In a first series of studies, effects of glutamate on cerebellar explants were determined. At physiological concentrations, glutamate is a neurotransmitter in the CNS of mammals, including humans. However, at sufficiently high concentrations, glutamate is neurotoxic, resulting in neuronal cell death. Because glutamate is a naturally occurring neurotransmitter in the CNS of mammals, including humans, and because glutamate neurotoxicity is recognized in the art as reflective of neurotoxicity in general, and including cell death and degeneration, it is a valuable tool useful for identifying and characterizing agents effective in treatment of neurodegeneration and neural cell death.

Materials and Methods

[0110] Cover slips were placed into a large Petri dish and washed in 70% alcohol for 5 minutes, then washed with Millipore H_2O. The cover slips were air dried, and coated with Poly-D-Lysine (1 mg/ml stock solution in PBS, 90-100μl)

for 2 hours at 34°C.

Extraction of Cerebellar Tissue

[0111] Postnatal day 8 Wistar rats were used for the study. The rats were sacrificed and placed in ice for 1 minute, decapitated and the cerebellum removed and placed on ice. Cerebellum tissue was placed in 1 ml of 0.65% glucose-supplemented PBS (10 μl 65% stock D (+)glucose/1ml PBS) in a large Petri dish, chopped up into smaller sections and triturated with a 1ml insulin syringe via a 23 G (0.4mm) needle, and then squirted back into the glucose solution in the large Petri dish. The tissue was sieved through (125μm pore size gauze) and centrifuged (2 minutes at 60g) twice to exchange the medium into serum-free BSA-supplemented START V medium (Biochrom, Germany). The second centrifugation step was done with 1ml of START V medium. The microexplants were reconstituted into 500μl of START V medium and put on ice.

Cultivation of Cerebellar Cells

[0112] Two hours after PDL-coating, the slides were washed with Millipore H_2O and air dried. Each slide was placed into a small Petri dish (diameter: 35 mm) and 40μl of START V/cell suspension was added. The tissue was incubated for 2 hours at 34°C (settlement period). START V-medium (1ml) was then added to the Petri dish and cultivated at 34° C in the presence of 5% CO_2 in air at 100% humidity for 48 hours.

Drug Application

[0113] For the study, certain explant cultures were exposed to vehicle (PBS) only. In the first study (Study 1) 10μl of toxin 1 (L-glutamate- 100mM in Millipore water; final concentration: 1mM) and 10μl of toxin 2 (3-nitropropionic acid-50mM- pH 7-in Millipore water, final concentration: 0.5mM) was applied simultaneously with the drug to be tested (10mM stock solution prepared in PBS and diluted to final concentrations between 1-100 nM). In each case, the drugs were left in contact with the explants for the duration of the study.

Methods for Determining Drug Effects

[0114] After explants were exposed to drugs for the study period, cells were then rinsed in PBS and then fixed in increasing concentrations of paraformaldehyde (500μl of 0.4% PFA was applied; then 1.2% PFA; then 3% PFA and finally 4% PFA (each fixation step: 2-3 minutes). Finally, the microexplants were rinsed in PBS.

[0115] Neurons in the explants were then evaluated for morphology (presence of neurites) and counted as live cells per microscopic field. Four fields displaying highest cell density were counted per cover slip and the data presented as mean ± standard error of the mean (SEM); n= 4 each. Statistical significance was evaluated by using the non-paired Student's t-test.

Results

Cyclic G-2-AllylP

[0116] The results of the study are shown in FIG.1. Glutamate treatment (1 mM; filled bar) resulted in about an 85% loss of cerebellar neurons having neurites compared to vehicle-treated controls (open bar). In contrast, cG-2AllylP significantly increased the numbers of cells having neurites in a dose-dependent manner when administered simultaneously with glutamate (shaded bars). Treatment with low doses of cG-2AllylP (100 pm to 10 nm) showed a significant decrease in glutamate-induced neurotoxicity.

Cyclic cyclopentyl-G-2MeP

[0117] The results of the study are shown in FIG. 2. Cyclic cyclopentyl-G-2MeP significantly increased the number of cells having neurites when simultaneously administered with glutamate (light shaded bars). Treatment with low doses of cyclic cyclopentyl-G-2MeP showed a significant decrease in glutamate-induced neurotoxicity.

Conclusions

[0118] Both cG-2AllylP and cyclic cyclopentyl-G-2MeP independently decreased or prevented glutamate-induced neurotoxicity, indicating that both drugs are neuroprotective and can be used to inhibit neuronal degeneration or cell death.

Example 6: Effects of cG-2AllylP on Hypoxic-Ischemic Injury I

Materials and Methods

[0119] To determine whether cG-2AllylP might prevent neuronal injury in response to stroke, cardiac arterial bypass graft surgery (CABG) or other hypoxic insults, a series of studies were carried out in rats that had been exposed to hypoxic-ischemic injury (HI). Adult rats (Wistar, 280-310g, male) were used. The modified Levine model preparation and experimental procedures were used (Rice et al, 1981, Ann. Neurol. :9: 131-141; Guan et al J., 1993, Cereb. Blood Flow Metab.: 13(4): 609-16). These procedures in brief, consist of an HI injury induced by unilateral carotid artery ligation followed by inhalational asphyxia in the animals with an implanted lateral ventricular cannula. A guide cannula was stereotaxically placed on the top of the dura 1.5mm to the right of the mid-line and 7.5mm anterior to the interaural zero plane under halothane anaesthesia. The right carotid artery was double ligated two days after the cannulation. After 1 hour recovery from the anaesthesia, each of the rats were placed in an incubator where the humidity (90±5%) and temperature (31°±0.5°C) were controlled for another hour, then exposed to hypoxia (6% oxygen) for 10 min. The animals were kept in the incubator for an additional 2 hours before treatment.

[0120] Nine pairs of rats were treated intracerebral ventricularly (icv) with either cG-2AllylP (2 ng) or its vehicle (normal saline) 2 hours after hypoxic-ischemic insult. Rats in each group were simultaneously infused with cG-2AllylP or its vehicle under light anaesthesia (1.5% halothane) 2 hours after the insult. A total volume of 20μl was infused (icv) over 20 minutes by a micro-infusion pump.

[0121] Histological examination was performed on rats 5 days after the hypoxic-ischemic injury. The rats were killed with an overdose of sodium pentobarbital and were perfused transcardially with normal saline followed by 10% formalin. The brains were kept in the same fixative for a minimum of 2 days before being processed using a standard paraffin imbedding procedure.

[0122] Coronal sections 8 μm in thickness were cut from the striatum, cerebral cortex and hippocampus and were stained with thionin and acid fuchsin. The histological outcome was assessed at three levels: (1) the mid level of the striatum, (2) where the completed hippocampus first appeared and (3) the level where the ventral horn of the hippocampus just appears. The severity of tissue damage was scored in the striatum, cortex and the CA1-2, CA3, CA4 and dentate gyrus of the hippocampus. Tissue damage was identified as neuronal loss (acidophilic (red) cytoplasm and contracted nuclei), pan-necrosis and cellular reactions. Tissue damage was scored using the following scoring system: 0: tissue showed no tissue damage, 1: <5% tissue was damaged, 2: <50% tissue was damaged, 3: >50% tissue was damaged and 4: >95% tissue was damaged.

Results and Conclusion

[0123] The results of this study are shown in FIG.3. FIG.3 shows that hypoxic-ischemic injury (left bars of each set) resulted in significant damage scores in each of the areas of the brain studied. FIG.3 also shows that central administration of a relatively low dose of cG-2AllylP (right bars of each set; 2 ng) significantly reduced the tissue damage in each brain region examined compared to the vehicle treated group ($p<0.001$).

[0124] It can be seen that cG-2AllylP can be neuroprotective against neural damage caused by hypoxic-ischemic injury, even when administered after hypoxic-ischemic injury. This surprising finding indicates that cG-2AllylP is a useful agent to treat a variety of conditions characterized by neural degeneration or cell death.

Example 7: Effects of cG-2AllylP on Hypoxic-Ischemic Injury II

Materials and Methods

[0125] Materials and methods described in Example 6 were used and the number of treatment groups was increased. Rats were divided into 5 treatment groups treated intracerebral ventricularly (icv) with one of 4 doses of cG-2AllylP or with its vehicle (normal saline) 2 hours after hypoxic-ischemic insult (1: n=10, 2ng; 2: n=9, 4 ng; 3: n=9, 20 ng; 4: n=10, 100 ng; and 5: n=9, vehicle).

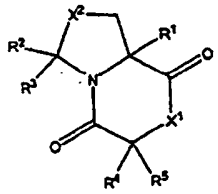
[0126] FIG. 4 shows hypoxia alone (vehicle) produces neuronal damage scores in all areas of the brain studied. In animals treated with cG-2allylP, hypoxia had less effect, even though the agent was administered after the hypoxic/ischemic injury. The neuroprotective effect was observed for all doses of cG-2allylP, except for the highest dose (100 ng) administered to the striatum. However, in all other sites and with all other doses, cG-2allylP lessened the neural damage effects of hypoxia/ischemia. Moreover, cG-2allylP had an increased efficacy in brain regions that experienced progressive injury associated with delayed cell death, such as that associated with apoptosis. In brain regions such as the dentate gyrus and the cerebral cortex, that are more resistant to HI injury, the progression of injury is known to be slower and more severe than in the brain regions that are more sensitive to HI injury such as the striatum and the CA1-2,

CA3 and CA4 sub-regions of the hippocampus. This result shows that cG-2allylP can be beneficial in treatment of chronic neurological disorders.

[0127] The descriptions and examples provided herein are for purposes of illustration only.

Claims

1. A compound having the formula:



or a pharmaceutically acceptable salt or hydrate thereof, wherein

X^1 is selected from the group consisting of NR', O and S;
X^2 is selected from the group consisting of CH_2, NR', O and S;
R^1 is selected from the group consisting of -OR',-SR',-NR'R',-NO_2, -CN,-C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl;
R^2, R^3, R^4 and R^5 are independently selected from the group consisting of -H, -OR', -SR', -NR'R', -NO_2, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl;
or R^4 and R^5 taken together are -CH_2-$(CH_2)_n$-CH_2- where n is an integer from 0-6;
or R^2 and R^3 taken together are -CH_2-$(CH_2)_n$-CH_2- where n is an integer from 0-6;
with the proviso that when R^1=methyl and R^2=R^3=R^4=H then $R^5 \neq$ benzyl; wherein a substituted alkyl, substituted heteroalkyl, substituted alkenyl, substituted alkynyl, substituted aryl, substituted heteroaryl or substituted arylalkyl is an alkyl, heteroalkyl, alkenyl, alkynyl, aryl, heteroaryl or arylalkyl radical wherein one or more of the hydrogen atoms are independently replaced with another substituent including -R',-OR',-SR',-NR'R',-NO_2, -CN,-C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R',-NR'-C(NR')-OR',-NR'-C(NR')-SR', NR'-C(NR')-NR'R', trihalomethyl and halogen where each R' is independently-H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl or heteroarylalkyl.

2. A compound according to claim 1 where R^1= methyl or allyl.

3. A compound according to claim 1 where R^2=R^3= methyl and X^2=S.

4. A compound according to claim 1 where R^1 = allyl, R^2=R^3=R^4=R^5=H, X^1=NH and X^2=CH_2 (cyclic G-2AllylP), or where R^1 = methyl, R^2= R^3 = H, R^4 and R^5 taken together are -CH_2-$(CH_2)_3$-CH_2-, X^1=NH and X^2=CH_2 (cyclic cyclohexyl G2MeP); or
where R^1 = methyl, R^2= R^3 = H, R^4 and R^5 taken together are -CH_2-$(CH_2)_2$-CH_2-, X^1=NH and X^2=CH_2 (cyclic cyclopentyl G2MeP).

5. A pharmaceutical composition comprising a compound as defined in any one of the preceding claims and a pharmaceutically acceptable excipient.

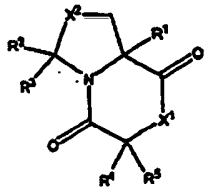
6. One or more compounds as defined in any one of claims 1 to 4 for use in protecting neurons otherwise destined to degenerate or die as a result of an injury or disease.

7. The one or more compounds for use of claim 6 wherein the disease is **characterized by** apoptotic neuronal death, necrotic neuronal cell death, neuronal cell degeneration, or wherein the injury results in necrotic neuronal cell death, apoptotic neuronal cell death, or neuronal cell degeneration.

8. The one or more compounds for use of claim 6 wherein said compound is selected from cyclic G-2AllylP, cyclic cyclohexyl-G-2MeP and cyclic cyclopentyl-G-2MeP.

Patentansprüche

1. Eine Verbindung mit der Formel:



oder ein pharmazeutisch verträgliches Salz oder Hydrat davon, worin

X^1 gewählt ist aus der Gruppe bestehend aus NR', O und S;
X^2 ist gewählt aus der Gruppe bestehend aus CH_2, NR', 0 und S;
R^1 ist gewählt aus der Gruppe bestehend aus -OR', -SR', -NR'R', $-NO_2$, -CN, -C(O)R', -(C(O)OR', -C(O)NR'R', -C(NR')NR'R', Trihalomethyl, Halogen, Alkyl, substituiertem Alkyl, Heteroalkyl, substituiertem Heteroalkyl, Alkenyl, substituiertem Alkenyl, Alkinyl, substituierem Alkinyl, Aryl, substituiertem Aryl, Heteroaryl, substituiertem Heteroaryl, Arylalkyl, substituiertem Arylalkyl, Heteroarylalkyl und substituiertem Heteroarylalkyl; jedes R' ist unabhängig gewählt aus der Gruppe bestehend aus -H, Alkyl, Heteroalkyl, Alkenyl, Alkinyl, Aryl, Arylalkyl, Heteroaryl und Heteroarylalkyl;
R^2, R^3, R^4 und R^5 sind unabhängig gewählt aus der Gruppe bestehend aus -H, -OR', -SR', -NR'R', $-NO_2$, -CN, -C(O)R',-C(O)OR', -C(O)NR'R', -C(NR')NR'R', Trihalomethyl, Halogen, Alkyl, substituiertem Alkyl, Heteroalkyl, substituiertem Heteroalkyl, Alkenyl, substituiertem Alkenyl, Alkinyl, substituiertem Alkinyl, Aryl, substituiertem Aryl, Heteroaryl, substituiertem Heteroaryl, Arylalkyl, substituiertem Arylalkyl, Heteroarylalkyl und substituiertem Heteroarylalkyl; jedes R' ist unabhängig gewählt aus der Gruppe bestehend aus -H, Alkyl, Heteroalkyl, Alkenyl, Alkinyl, Aryl, Arylalkyl, Heteroaryl und Heteroarylalkyl;
oder R^4 und R^5 zusammengenommen sind $-CH_2-(CH_2)_n-CH_2-$, worin n eine ganze Zahl von 0-6 ist;
oder R^2 und R^3 zusammengenommen sind $-CH_2-(CH_2)_n-CH_2-$, worin n eine ganze Zahl von 0-6 ist;
unter der Voraussetzung, dass, wenn R^1=Methyl ist und $R^2=R^3=R^4$=H, dann ist $R^5 \neq$ Benzyl; worin ein substituiertes Alkyl, substituiertes Heteroalkyl, substituiertes Alkenyl, substituiertes Alkinyl, substituiertes Aryl, substituiertes Heteroaryl oder substituiertes Arylalkyl ein Alkyl-, Heteroalkyl-, Alkenyl-, Alkinyl-, Aryl-, Heteroaryl- oder Arylalkylradikal ist, worin eines oder mehrere der Wasserstoffatome unabhängig ersetzt sind durch einen anderen Substituenten einschließlich -R', -OR', -SR', -NR'R', $-NO_2$, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', -NR'-C(NR')-OR', -NR'-C(NR')-SR', -NR'-C(NR')-NR'R', Trihalomethyl und Halogen, worin jedes R' unabhängig -H, Alkyl, Heteroalkyl, Alkenyl, Alkinyl, Aryl, Arylalkyl, Heteroaryl oder Heteroarylalkyl ist.

2. Eine Verbindung gemäß Anspruch 1, worin R^1=Methyl oder Allyl ist.

3. Eine Verbindung gemäß Anspruch 1, worin $R^2=R^3$=Methyl und X^2=S ist.

4. Eine Verbindung gemäß Anspruch 1, worin R^1=Allyl, $R^2=R^3=R^4=R^5$=H, X^1=NH und $X^2=CH_2$ (zyklisches G-2AllylP) ist, oder
worin R'=Methyl, $R^2=R^3$=H, R^4 und R^5 zusammengenommen sind $-CH_2-(CH_2)_3-CH_3-$, X^1=NH und $X^2=CH_2$ (zyklisches Cyclohexyl G2MeP); oder
worin R^1=Methyl, $R^2=R^3$=H, R^4 und R^5 zusammengenommen sind $-CH_2-(CH_2)_2-CH_2-$, X^1=NH und $X^2=CH_2$ (zyklisches Cyclopentyl G2MeP).

5. Eine pharmazeutische Zusammensetzung umfassend eine Verbindung wie definiert in irgendeinem der vorhergehenden Ansprüche und ein pharmazeutisch verträgliches Hilfsmittel.

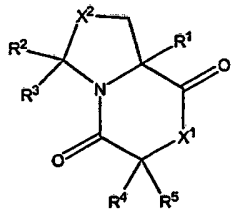
6. Eine oder mehrere Verbindungen wie definiert in irgendeinem der Ansprüche 1 bis 4 für die Verwendung im Schutz von Neuronen, die sonst bestimmt sind zu degenerieren oder zu sterben als Folge einer Verletzung oder Krankheit.

7. Die eine oder die mehreren Verbindungen für die Verwendung gemäß Anspruch 6, worin die Krankheit durch apoptotischen Neuronentod, nekrotischen neuronalen Zelltod, neuronale Zelldegeneration gekennzeichnet ist, oder worin die Verletzung zu nekrotischem neuronalen Zelltod, apoptotischem neuronalen Zelltod oder neuronaler Zelldegeneration führt.

8. Die eine oder die mehreren Verbindungen für die Verwendung gemäß Anspruch 6, worin die Verbindung gewählt ist aus zyklischem G-2AllylP, zyklischem Cyclohexyl-G-2MeP und zyklischem Cyclopentyl-G-2MeP.

Revendications

1. Composé de formule :



ou un sel ou hydrate pharmaceutiquement acceptable d'un tel composé,
formule dans laquelle

X^1 est choisi dans le groupe constitué par NR', O et S ;
X^2 est choisi dans le groupe constitué par CH_2, NR', O et S ;
R^1 est choisi dans le groupe constitué par -OR',-SR', -NR'R', $-NO_2$, -CN, -C(O)R', -C(O)OR',-C(O)NR'R', -C(NR')NR'R', les halogènes, et les radicaux trihalogénométhyle, alkyle, alkyle substitué, hétéroalkyle, hétéroalkyle substitué, alcényle, alcényle substitué, alcynyle, alcynyle substitué, aryle, aryle substitué, hétéroaryle, hétéroaryle substitué, arylalkyle, arylalkyle substitué, hétéroarylalkyle et hétéroarylalkyle substitué ; chaque R' est indépendamment choisi dans le groupe constitué par -H et les radicaux alkyle, hétéroalkyle, alcényle, alcynyle, aryle, arylalkyle, hétéroaryle et hétéroarylalkyle ;
R^2, R^3, R^4 et R^5 sont indépendamment choisis dans le groupe constitué par -H, -OR', -SR', -NR'R', $-NO_2$, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', les halogènes, et les radicaux trihalogénométhyle, alkyle, alkyle substitué, hétéroalkyle, hétéroalkyle substitué, alcényle, alcényle substitué, alcynyle, alcynyle substitué, aryle, aryle substitué, hétéroaryle, hétéroaryle substitué, arylalkyle, arylalkyle substitué, hétéroarylalkyle et hétéroarylalkyle substitué ; chaque R' est indépendamment choisi dans le groupe constitué par -H et les radicaux alkyle, hétéroalkyle, alcényle, alcynyle, aryle, arylalkyle, hétéroaryle et hétéroarylalkyle ;
ou bien R^4 et R^5, pris ensemble, forment $-CH_2-(CH_2)_n-CH_2-$ où n est un entier de 0 à 6 ;
ou bien R^2 et R^3, pris ensemble, forment $-CH_2-(CH_2)_n-CH_2-$ où n est un entier de 0 à 6 ;
sous réserve que, lorsque R^1 = méthyle et $R^2 = R^3 = R^4$ = H, alors $R^5 \neq$ benzyle ;
où l'alkyle substitué, hétéroalkyle substitué, alcényle substitué, alcynyle substitué, aryle substitué, hétéroaryle substitué ou arylalkyle substitué est un radical alkyle, hétéroalkyle, alcényle, alcynyle, aryle, hétéroaryle ou arylalkyle dont un ou plusieurs atomes d'hydrogène sont indépendamment remplacés par un autre substituants comprenant -R', -OR', -SR', -NR'R',$-NO_2$, -CN, -C(O)R', -C(O)OR', -C(O)NR'R',-C(NR')NR'R', -NR'-C(NR')-OR', -NR'-C(NR')-SR', -NR'-C(NR')-NR'R', les halogènes et les radicaux trihalogénométhyle, où chaque R' est indépendamment -H ou un radical alkyle, hétéroalkyle, alcényle, alcynyle, aryle, arylalkyle, hétéroaryle et hétéroarylalkyle.

2. Composé selon la revendication 1, dans lequel R^1 = méthyle ou allyle.

3. Composé selon la revendication 1, dans lequel R^2 =R^3 = méthyle et X^2 = S.

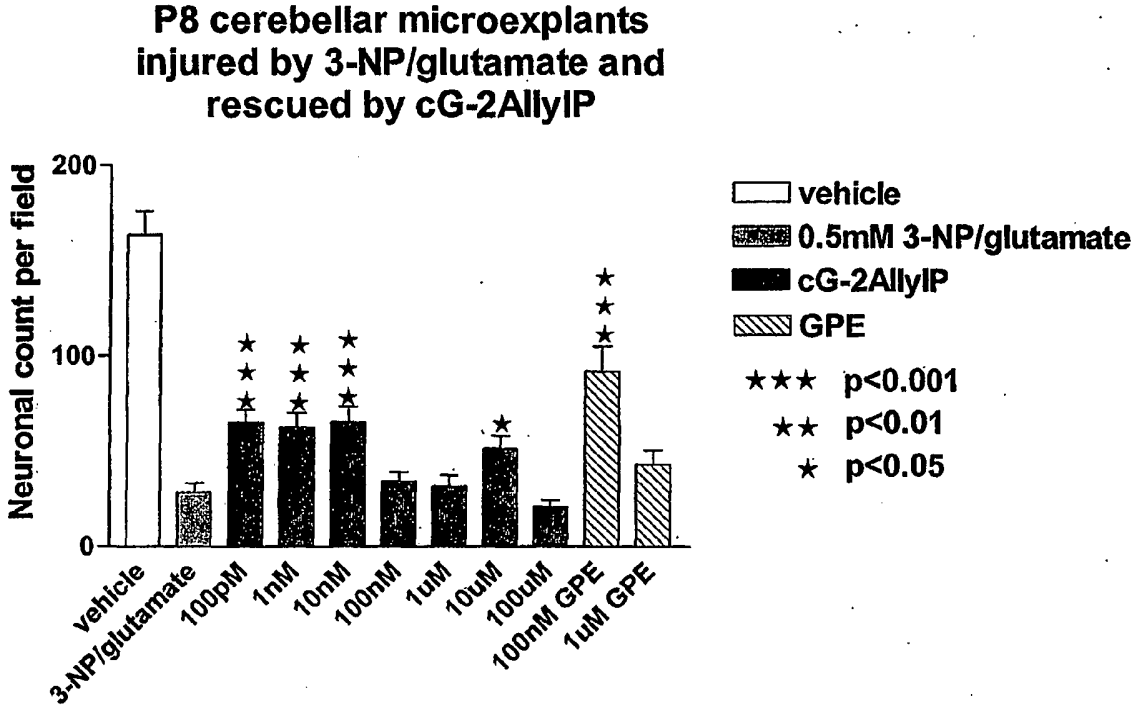
4. Composé selon la revendication 1,
dans lequel R^1 = allyle, R^2 = R^3 = R^4 = R^5 = H, X^1 = NH et X^2 = CH_2 (G-2AllylP cyclique), ou
dans lequel R^1 = méthyle, R^2 = R^3 = H, R^4 et R^5 pris ensemble forment -CH_2-$(CH_2)_3$-CH_2-, X^1 = NH et X^2 = CH_2 (cyclohexyl-G2MeP cyclique) ; ou
dans lequel R^1 = méthyle, R^2 = R^3 = H, R^4 et R^5 pris ensemble forment -CH_2-$(CH_2)_2$-CH_2-, X^1 = NH et X^2 = CH_2 (cyclopentyl-G2MeP cyclique).

5. Composition pharmaceutique comprenant un composé tel que défini dans l'une quelconque des revendications précédentes et un excipient pharmaceutiquement acceptable.

6. Un ou plusieurs composés tels que définis dans l'une quelconque des revendications 1 à 4 pour utilisation dans la protection de neurones sinon destinés à dégénérer ou à mourir en résultat d'une lésion ou d'une maladie.

7. Le ou les composés pour utilisation selon la revendication 6, dans lesquels la maladie est **caractérisée par** une mort neuronale apoptotique, une mort de cellules neuronales nécrotique, une dégénérescence de cellules neuronales, ou dans lesquels la lésion a pour résultat une mort de cellules neuronales nécrotique, une mort de cellules neuronales apoptotique, ou une dégénérescence de cellules neuronales.

8. Le ou les composés pour utilisation selon la revendication 6, dans lesquels ledit composé est choisi parmi le G-2AllylP cyclique, cyclohexyl-G-2MeP cyclique et le cyclopentyl-G2MeP cyclique.



Cell number analysed after 48hrs. Between 100pM to 10nM cG-2AllylP (N=4) the recovery from injury is in between 25.5% to 27.3%. 100nM GPE displays 47.1% recovery.

Figure 1

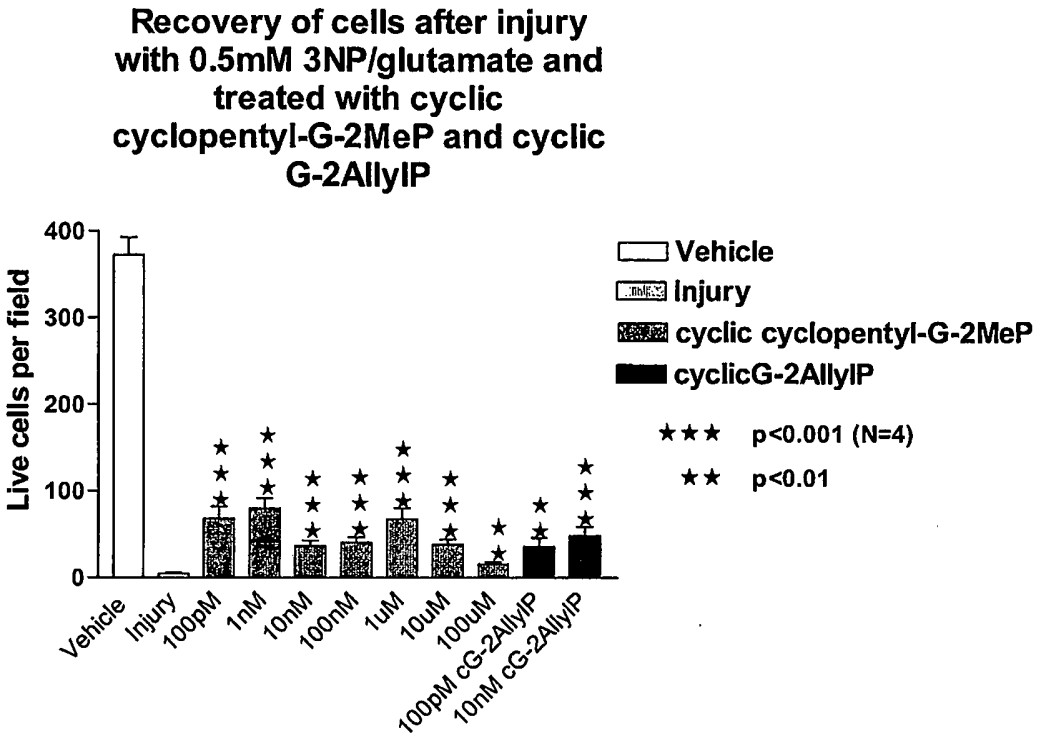

Recovery of cells after injury with 0.5mM 3NP/glutamate and treated with cyclic cyclopentyl-G-2MeP and cyclic G-2AllylP
Live cells per field
400
300
200
100
0
Vehicle
Injury
100pM
1nM
10nM
100nM
1uM
10uM
100uM
100pM cG-2AllylP
10nM cG-2AllylP
Vehicle
Injury
cyclic cyclopentyl-G-2MeP
cyclicG-2AllylP
★★★ p<0.001 (N=4)
★★ p<0.01
Cells are fixed after 48hrs

Figure 2

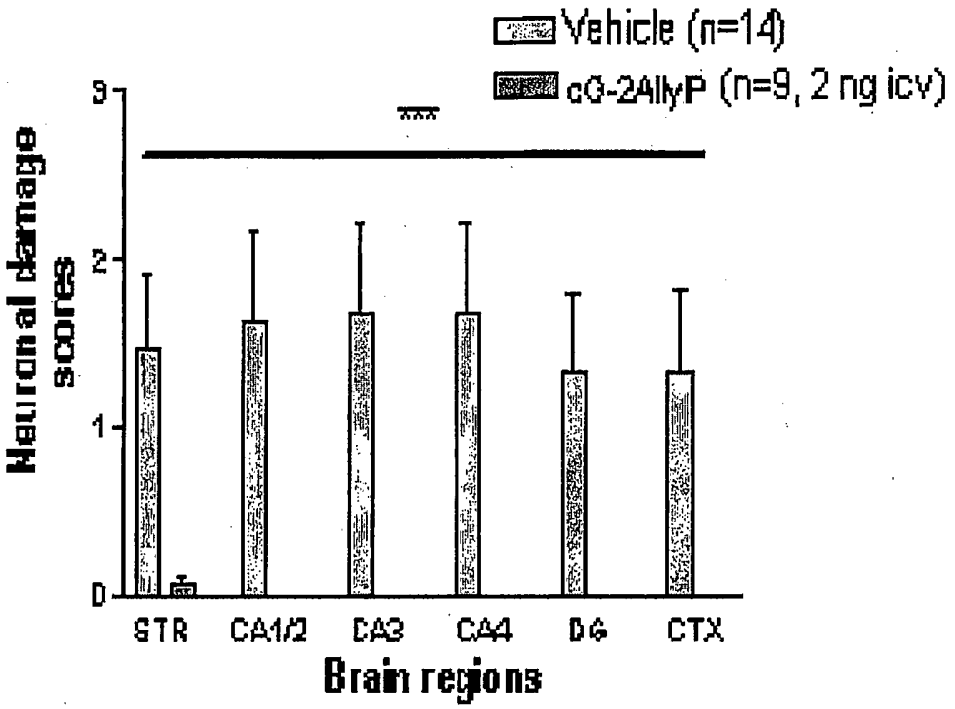

Vehicle (n=14)
cG-2AllyP (n=9, 2 ng icv)
****
Neuronal damage scores
0
1
2
3
STR
CA1/2
CA3
CA4
DG
CTX
Brain regions

Figure 3

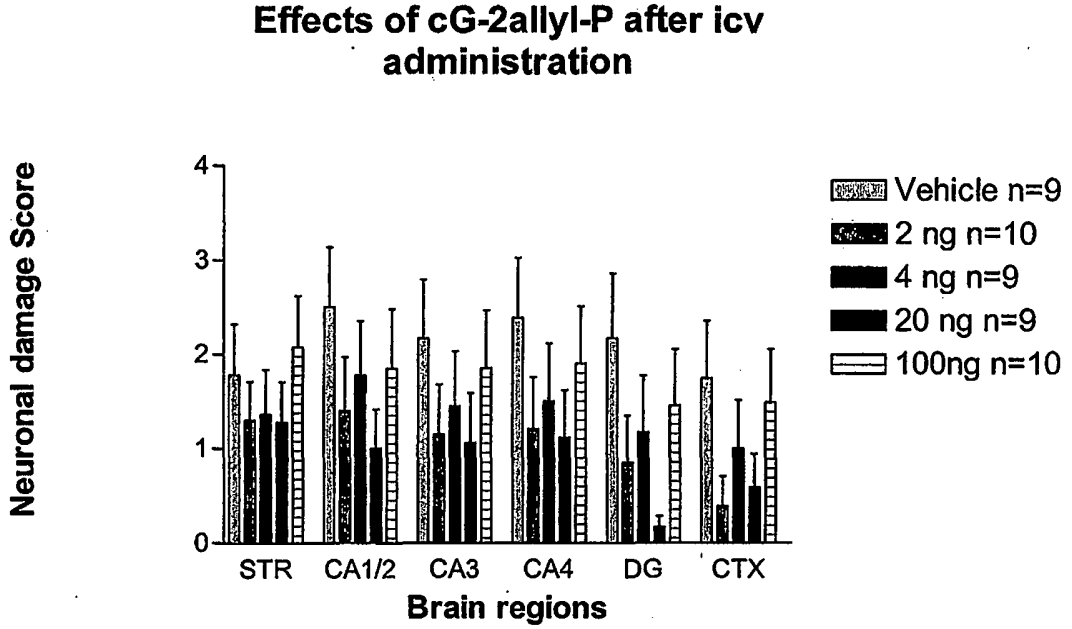

Effects of cG-2allyl-P after icv administration
Neuronal damage Score
0
1
2
3
4
STR
CA1/2
CA3
CA4
DG
CTX
Brain regions
Vehicle n=9
2 ng n=10
4 ng n=9
20 ng n=9
100ng n=10

Figure 4

REFERENCES CITED IN THE DESCRIPTION

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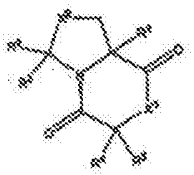
SZABADALMI IGÉNYPONTOK

1. Vegyület, amelynek képlete



vagy ennek gyógyszerészetileg alkalmazható sója vagy hidrátja, ahol

X^1 jelentése NR', O és S csoportból megválasztott;

X^2 jelentése CH_2, NR', O és S csoportból megválasztott;

R^1 jelentése -OR', -SR', -NR'R', $-NO_2$, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalometil, halogén, alkil, szubsztituált alkil, heteroalkil, szubsztituált heteroalkil, alkenil, szubsztituált alkenil, alkinil, szubsztituált alkinil, aril, szubsztituált aril, heteroaril, szubsztituált heteroaril, arilalkil, szubsztituált arilalkil, heteroarilalkil és szubsztituált heteroarilalkil csoportból megválasztott; minden R' jelentése egyenként és egymástól függetlenül -H, alkil, heteroalkil, alkenil, alkinil, aril, arilalkil, heteroaril és heteroarilalkil csoportból megválasztott;

R^2, R^3, R^4 és R^5 jelentése egymástól függetlenül -H, -OR', -SR', -NR'R', $-NO_2$, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalometil, halogén, alkil, szubsztituált alkil, heteroalkil, szubsztituált heteroalkil, alkenil, szubsztituált alkenil, alkinil, szubsztituált alkinil, aril, szubsztituált aril, heteroaril, szubsztituált heteroaril, arilalkil, szubsztituált arilalkil, heteroarilalkil és szubsztituált heteroarilalkil csoportból megválasztott; minden R' jelentése egyenként és egymástól függetlenül -H, alkil, heteroalkil, alkenil, alkinil, aril, arilalkil, heteroaril és heteroarilalkil csoportból megválasztott;

vagy R^4 és R^5 jelentése együtt $-CH_2-(CH_2)_n-CH_2-$ ahol n értéke 0-6 egész szám;

vagy R^2 és R^3 jelentése együtt $-CH_2-(CH_2)_n-CH_2-$ ahol n értéke 0-6 egész szám;

azzal a megszorítással, hogy ha R^1=metil és R^2=R^3=R^4=H, akkor $R^5 \neq$ benzil;

ahol a szubsztituált alkil, szubsztituált heteroalkil, szubsztituált alkenil, szubsztituált alkinil, szubsztituált aril, szubsztituált heteroaril vagy szubsztituált arilalkil olyan alkil, heteroalkil, alkenil, alkinil, aril, heteroaril vagy arilalkil csoport, ahol egy vagy több hidrogénatom helyett egymástól függetlenül más szubsztituens áll, amely -R', -OR', -SR', -NR'R', $-NO_2$, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', -NR'-C(NR')-OR', -NR'-C(NR')-SR', NR'-C(NR')-NR'R', trihalometil és halogén csoportból megválasztott, ahol minden R' jelentése egyenként és egymástól függetlenül -H, alkil, heteroalkil, alkenil, alkinil, aril, arilalkil, heteroaril vagy heteroarilalkil.

2. Az 1. igénypont szerinti vegyület, ahol R^1= metil vagy allil.

3. Az 1. igénypont szerinti vegyület, ahol R^2=R^3= metil és X^2=S.

4. Az 1. igénypont szerinti vegyület, ahol R^1 = allil, R^2=R^3=R^4=R^5= H, X^1= NH és X^2= CH_2 (ciklusos G-2AllilP), vagy
ahol R^1 = metil, R^2= R^3 = H, R^4 és R^5 jelentése együtt -CH_2-$(CH_2)_2$-CH_2-, X^1= NH és X^2= CH_2 (ciklusos ciklohexil G2MeP); vagy
ahol R^1 = metil, R^2= R^3 = H, R^4 és R^5 jelentése együtt -CH_2-$(CH_2)_2$-CH_2-, X^1= NH és X^2= CH_2 (ciklusos ciklopentil G2MeP).

5. Gyógyszerkészítmény, amely tartalmazza az előző igénypontok bármelyike szerinti vegyületet és gyógyszerészetileg alkalmazható segédanyagot.

6. Az 1-4. igénypontok bármelyike szerinti egy vagy több vegyület sérülés vagy betegség miatt degenerálódásra vagy pusztulásra rendelt neuronok védelmében történő alkalmazásra.

7. A 6. igénypont szerinti egy vagy több vegyület az adott alkalmazásra, ahol a betegség jellemzője az apoptotikus idegsejt pusztulás, nekrotikus idegsejt pusztulás, idegsejt degeneráció, vagy ahol a sérülés nekrotikus idegsejt pusztulást, apoptotikus idegsejt pusztulást vagy idegsejt degenerációt vált ki.

8. A 6. igénypont szerinti egy vagy több vegyület az adott alkalmazásra, ahol az említett vegyület ciklusos G-2AllilP, ciklusos ciklohexil-G-2MeP és ciklusos ciklopentil-G-2MeP közül megválasztott.